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Please perform a search on the attached claims. Keywords and examples are provided. Please include Agricultural & Veterinary databases, US & foreign patent databases, Dissertation abstracts, Inside conferences, Medline, Biosis, EMBASE, PASCAL, JICSTE PLUS, Derwent, FEDRIP, CRIS/USDA, TOXLINE & File 53.

Inventors: MICHAEL A. APICELLA
MELVIN G. SUNSHINE
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Date completed: 03-01-99

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___ N.A. Sequence
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PTO-1590 (9-90)

Devi, S.
09/077572

01mar99 10:58:30 User219783 Session D1447.2

SYSTEM:OS - DIALOG OneSearch
File 440:Current Contents Search(R) 1990-1999/Mar W1
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*File 440: Records starting 1997 to 1998W3 were reloaded, please note the changed in accession numbers.
File 71:ELSEVIER BIOBASE 1994-1999/Jan W4
(c) 1999 Elsevier Science B.V.
File 144:Pascal 1973-1999/Jan
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Set	Items	Description

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- key terms

Set	Items	Description
S1	74	(HTRB OR HTR(W)B) AND (GRAM(W)(NEGATIVE OR NEG) OR SALMONELL? OR COLI OR HAEMOPHIL? OR HEMOPHIL?)
S2	28	RD (unique items)
S3	26	S2 AND (MUTANT? ? OR MUTAGEN? OR MUTAT? OR POLYMORPH? OR POLY(W)MORPHI???)

? t 3/3,ab/1-26

3/3,AB/1 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09785010 GENUINE ARTICLE#: 113PM NUMBER OF REFERENCES: 49
TITLE: A lethal role for lipid A in **Salmonella** infections
AUTHOR(S): Khan SA; Everest P; Servos S; Foxwell N; Zahringer U; Brade H; Rietschel ET; Dougan G; Charles IG; Maskell DJ (REPRINT)
CORPORATE SOURCE: UNIV CAMBRIDGE,DEPT CLIN VET MED, CTR VET SCI, MADINGLEY RD/CAMBRIDGE CB3 0ES//ENGLAND/ (REPRINT); UNIV CAMBRIDGE,DEPT CLIN VET MED, CTR VET SCI/CAMBRIDGE CB3 0ES//ENGLAND/; UNIV LONDON IMPERIAL COLL

SCI TECHNOL & MED,DEPT BIOCHEM/LONDON SW7 2AY//ENGLAND/; UNIV LONDON UNIV COLL,RAYNE INST, WOLFSON INST BIOMED RES/LONDON WC1E 6JJ//ENGLAND/ ; BORSTEL RES CTR,CTR MED & BIOSCI/D-23845 BORSTEL//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1998, V29, N2 (JUL), P571-579
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: **Salmonella** infections in naturally susceptible mice grow rapidly, with death occurring only after bacterial numbers in vivo have reached a high threshold level, commonly called the lethal load. Despite much speculation, no direct evidence has been available to substantiate a role for any candidate bacterial components in causing death. One of the most likely candidates for the lethal toxin in **salmonellosis** is endotoxin, specifically the lipid A domain of the lipopolysaccharide (LPS) molecule. Consequently, we have constructed a **Salmonella mutant** with a deletion-insertion in its waaN gene, which encodes the enzyme that catalyses one of the two secondary acylation reactions that complete lipid A biosynthesis, The **mutant** biosynthesizes a lipid A molecule racking a single fatty acyl chain and is consequently less able to induce cytokine and inducible nitric oxide synthase (iNOS) responses both in vivo and in vitro. The **mutant** bacteria appear healthy, are not sensitive to increased growth temperature and synthesize a full-length O-antigen-containing LPS molecule lacking only the expected secondary acyl chain. On intravenous inoculation into susceptible BALB/c mice, wild-type **salmonellae** grew at the expected rate of approximately 10-fold per day in livers and spleens and caused the death of the infected mice when lethal loads of approximately 10(8) were attained in these organs. Somewhat unexpectedly, waaN **mutant** bacteria grew at exactly the same rate as wild-type bacteria in BALB/c mice but, when counts reached 10(8) per organ, mice infected with **mutant** bacteria survived. Bacterial growth continued until unprecedentedly high counts of 10(9) per organ were attained, when approximately 10% of the mice died. Most of the animals carrying these high bacterial loads survived, and the bacteria were slowly cleared from the organs. These experiments provide the first direct evidence that death in a mouse typhoid infection is directly dependent on the toxicity of lipid A and Suggest that this may be mediated via proinflammatory cytokine and/or iNOS responses.
ISSN: 0950-382X

09654995 GENUINE ARTICLE#: ZZ982 NUMBER OF REFERENCES: 56

TITLE: Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH:Ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by Pseudomonas fluorescens WCS365

AUTHOR(S): Dekkers LC (REPRINT); vanderBij AJ; Mulders IHM; Phoelich CC; Wentwoord RAR; Glandorf DCM; Wijffelman CA; Lugtenberg BJJ

CORPORATE SOURCE: LEIDEN UNIV,INST MOL PLANT SCI, CLUSIUS LAB, WASSENAARSEWEG 64/NL-2333 AL LEIDEN//NETHERLANDS/ (REPRINT); UNIV UTRECHT,DEPT PLANT ECOL & EVOLUTIONARY BIOL/NL-3508 TB UTRECHT//NETHERLANDS/

PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR PLANT-MICROBE INTERACTIONS, 1998, V11, N8 (AUG), P 763-771

PUBLISHER: AMER PHYTOPATHOLOGICAL SOC, 3340 PILOT KNOB ROAD, ST PAUL, MN 55121

ISSN: 0894-0282

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Colonization-defective, transposon-induced **mutants** of the efficient root colonizer Pseudomonas fluorescens WCS365 were identified with a gnotobiotic system. Most **mutants** were impaired in known colonization traits, i.e., prototrophy for amino acids, motility, and synthesis of the O-antigen of LPS (lipopolysaccharide). **Mutants** lacking the O-antigen of LPS were impaired in both colonization and competitive growth whereas one **mutant** (PCL1205) with a shorter O-antigen chain was defective only in colonization ability, suggesting a role for the intact O-antigen of LPS in colonization. Eight competitive colonization **mutants** that were not defective in the above-mentioned traits colonized the tomato root tip well when inoculated alone, but were defective in competitive root colonization of tomato, radish, and wheat, indicating they contained **mutations** affecting host range. One of these eight **mutants** (PCL1201) was further characterized and contains a **mutation** in a gene that shows homology to the Escherichia **coli** nuo4 gene, which encodes a subunit of one of two known NADH:ubiquinone oxidoreductases. Competition experiments in an oxygen-poor medium between **mutant** PCL1201 and its parental strain showed a decreased growth rate of **mutant** PCL1201, The requirement of the nuo4 gene homolog for optimal growth under conditions of oxygen limitation suggests that the root-tip environment is micro-aerobic. A **mutant** characterized by a slow growth rate (PCL1216) was analyzed further and contained a **mutation** in a gene with similarity to the E. **coli** **HtrB** protein, a lauroyl transferase that functions in lipid A biosynthesis.

ISSN: 0894-0282

09475100 GENUINE ARTICLE#: ZN277 NUMBER OF REFERENCES: 53
TITLE: Function of Escherichia **coli** MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis
AUTHOR(S): Zhou ZM; White KA; Polissi A; Georgopoulos C; Raetz CRH (REPRINT)
CORPORATE SOURCE: DUKE UNIV,MED CTR, DEPT BIOCHEM, POB 3711/DURHAM//NC/27710 (REPRINT); DUKE UNIV,MED CTR, DEPT BIOCHEM/DURHAM//NC/27710; CTR MED UNIV GENEVA,DEPT BIOCHIM MED/CH-1211 GENEVA 4//SWITZERLAND/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BIOLOGICAL CHEMISTRY, 1998, V273, N20 (MAY 15), P 12466-12475
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
ISSN: 0021-9258
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The Escherichia **coli** msbA gene, first identified as a multicopy suppressor of **htrB mutations**, has been proposed to transport nascent core-lipid A molecules across the inner membrane (Polissi, k, and Georgopoulos, C. (1996) Mol. Microbiol. 20, 1221-1233), msbA is an essential E. **coli** gene with high sequence similarity to mammalian Mdr proteins and certain types of bacterial ABC transporters, **htrB** is required for growth above 32 degrees C and encodes the lauroyltransferase that acts after Kdo addition during lipid A biosynthesis (Clementz, T,, Bednarski, J., and Raetz, C. R. H. (1996) J. Biol. Chem. 271, 12095-12102). By using a quantitative new P-32(i) labeling technique, we demonstrate that hexa-acylated species of lipid A predominate in the outer membranes of wild type E. **coli** labeled for several generations at 42 degrees C. In contrast, in **htrB mutants** shifted to 42 degrees C for 3 h, tetraacylated lipid A species and glycerophospholipids accumulate in the inner membrane. Extra copies of the cloned msbA gene restore the ability of **htrB mutants** to grow at 42 degrees C, but they do not increase the extent of lipid A acylation. However, a significant fraction of the tetraacylated lipid A species that accumulate in **htrB mutants** are transported to the outer membrane in the presence of extra copies of msbA. E. **coli** strains in which msbA synthesis is selectively shut off at 42 degrees C accumulate hexa-acylated lipid A and glycerophospholipids in their inner membranes. Our results support the view that MsbA plays a role in lipid A and possibly glycerophospholipid transport. The tetra-acylated lipid A precursors that accumulate in **htrB mutants** may not be transported as efficiently by MsbA as are penta- or hexaacylated lipid A species.
ISSN: 0021-9258

09035667 GENUINE ARTICLE#: YL266 NUMBER OF REFERENCES: 23
TITLE: Temperature-sensitive lesions in the Francisella novicida valA gene cloned into an Escherichia **coli** msbA lpxK **mutant** affecting deoxycholate resistance and lipopolysaccharide assembly at the restrictive temperature
AUTHOR(S): McDonald MK; Cowley SC (REPRINT); Nano FE
CORPORATE SOURCE: UNIV VICTORIA,DEPT BIOCHEM & MICROBIOL, PETCH BLDG/VICTORIA/BC V8W 3P6/CANADA/ (REPRINT); UNIV VICTORIA,DEPT BIOCHEM & MICROBIOL/VICTORIA/BC V8W 3P6/CANADA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1997, V179, N24 (DEC), P7638-7643
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0021-9193
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The valAB locus of Francisella novicida has previously been found to be highly similar at the deduced amino acid level to msbA lpxK of Escherichia **coli**, Both ValA and MsbA are members of the superfamily of ABC transporters, and they appear to have similar functions, In this study we describe the isolation of a temperature-sensitive valAB locus, DNA sequence analysis indicates that the only changes to the ValAB deduced amino acid sequence are changes of S453 to an F and T458 to an I in ValA. E. coil strains defective in msbA and expressing temperature-sensitive ValA rapidly ceased growth when shifted from a permissive temperature to a restrictive temperature, After 1 h at the restrictive temperature, cells were much moire sensitive to deoxycholate treatment, To test the hypothesis that ValA is responsible for the transport or assembly of lipopolysaccharide, we introduced gseA, a Kdo (3-deoxy-D-manno-octulosonic acid) transferase from Chlamydia trachomatis, into a strain with a temperature-sensitive valA allele and a nonfunctional msbA locus, These recombinants were defective in cell surface expression of the chlamydial genus-specific epitope within 15 min of a shift to the nonpermissive temperature. Also, there was enhanced association of the epitope with the inner membrane after a shift to the nonpermissive temperature. Thus, we propose that ValA is involved in the transport of lipopolysaccharide to the outer membrane.
ISSN: 0021-9193

3/3,AB/5 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08936093 GENUINE ARTICLE#: YD176 NUMBER OF REFERENCES: 70
TITLE: Study of the role of the **htrB** gene in **Salmonella** typhimurium virulence

AUTHOR(S): Jones BD (REPRINT); Nichols WA; Gibson BW; Sunshine MG; Apicella MA
CORPORATE SOURCE: UNIV IOWA,COLL MED, DEPT MICROBIOL/IOWA CITY//IA/52242 (REPRINT); UNIV CALIF SAN FRANCISCO,SCH PHARM, DEPT PHARMACEUT CHEM/SAN FRANCISCO//CA/94143
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N11 (NOV), P4778-4783
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: We have undertaken a study to investigate the contribution of the **htrB** gene to the virulence of pathogenic **Salmonella** typhimurium. An **htrB**::mini-Tn10 **mutation** from Escherichia call was transferred by transduction to the mouse-virulent strain S. typhimurium SL1344 to create an **htrB mutant**. The S.typhimurium **htrB mutant** was inoculated into mice and found to be severely limited in its ability to colonize organs of the lymphatic system and to cause systemic disease in mice. A variety of experiments were performed to determine the possible reasons for this loss of virulence. Serum killing assays revealed that the S. typhimurium **htrB mutant** was as resistant to killing by complement as the wild-type strain. However, macrophage survival assays revealed that the S. typhimurium **htrB mutant**,vas more sensitive to the intracellular environment of murine macrophages than the wild-type strain. In addition, the bioactivity of the lipopolysaccharide (LPS) of the **htrB mutant** was reduced compared to that of the LPS from the parent strain as measured by both a Limulus amoebocyte lysate endotoxin quantitation assay and a tumor necrosis factor alpha bioassay. These results indicate that the **htrB** gene plays a role in the virulence of S. typhimurium.
ISSN: 0019-9567

3/3,AB/6 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08936045 GENUINE ARTICLE#: YD176 NUMBER OF REFERENCES: 30
TITLE: Evaluation of the virulence of nontypeable **Haemophilus** influenzae lipooligosaccharide **htrB** and rfaD **mutants** in the chinchilla model of otitis media
AUTHOR(S): DeMaria TF (REPRINT); Apicella MA; Nichols WA; Leake ER
CORPORATE SOURCE: OHIO STATE UNIV,COLL MED, DIV OTOL RES, ROOM 4331 UHC, 456 W 10TH AVE/COLUMBUS//OH/43210 (REPRINT); UNIV IOWA,/IOWA CITY//IA/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N11 (NOV), P4431-4435
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171

ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Considerable evidence has implicated nontypeable **Haemophilus** influenzae (NTHi) lipooligosaccharide (LOS) in the pathogenesis of otitis media (OM); however, its exact role has not been conclusively established, Recently, two NTHi LOS-deficient **mutants** have been created and described. Strain 2019-DK1, an rfaD gene **mutant**, expresses a truncated LOS consisting of only three deoxy-D-manno-octulosonic acid residues, a single heptose, and lipid A, Strain 2019-B29, an isogenic **htrB mutant**, possesses an altered oligosaccharide core and an altered lipid A, Each strain's ability to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation was evaluated in the chinchilla model. Nasopharyngeal colonization data indicate that the parent strain and both **mutants** are able to colonize the nasopharynx and exhibit comparable clearance kinetics. Compared with the parent and each other, however, the **mutants** demonstrated marked differences in virulence regarding their relative abilities to induce OM and persist in the middle ear post-transbullar inoculation, Strain B29 required a 3-log-greater dose to induce OM than the parent strain and did not exhibit evidence of sustained multiplication but persisted for the same duration as the parent, Conversely, strain-DK1, even when inoculated at a dose 4 logs greater than the parent dose, was eliminated from the middle ear 72 h after challenge, A comparison of the relative pathogenicities of these isolates provides the opportunity to address fundamental questions regarding the contribution of LOS to pathogenesis issues at the molecular level, Specifically, the impact of these LOS gene disruptions on OM pathogenesis can be defined and may thus provide potential new targets for future protection and intervention strategies.
ISSN: 0019-9567

08773455 GENUINE ARTICLE#: XT829 NUMBER OF REFERENCES: 28
TITLE: **htrB** of **Haemophilus** influenzae: determination of biochemical activity and effects on virulence and lipooligosaccharide toxicity
AUTHOR(S): Nichols WA; Raetz CRH; Clementz T; Smith AL; Hanson JA; Ketterer MR; Sunshine M; Apicella MA (REPRINT)
CORPORATE SOURCE: UNIV IOWA,COLL MED, DEPT MICROBIOL BSB 3 403, 51 NEWTON RD/IOWA CITY//IA/52242 (REPRINT); UNIV IOWA,COLL MED, DEPT MICROBIOL BSB 3 403/IOWA CITY//IA/52242; DUKE UNIV,MED CTR, DEPT BIOCHEM/DURHAM//NC/27710; UNIV MISSOURI,SCH MED, DEPT MOL MICROBIOL & IMMUNOL/COLUMBIA//MO/65212
PUBLICATION TYPE: JOURNAL

PUBLICATION: JOURNAL OF ENDOTOXIN RESEARCH, 1997, V4, N3 (JUN), P163-172
PUBLISHER: CHURCHILL LIVINGSTONE, JOURNAL PRODUCTION DEPT, ROBERT STEVENSON HOUSE, 1-3 BAXTERS PLACE, LEITH WALK, EDINBURGH EH1 3AF, MIDLOTHIAN, SCOTLAND
ISSN: 0968-0519
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The **htrB mutant** of **Haemophilus** influenzae (strain B29) has been shown to lack secondary (nonhydroxylated) acyl groups in its lipid A. We have determined through in vitro biochemical assays that the **HtrB** protein acts as a specific acyltransferase in the late stages of lipid A biosynthesis and that the preferred acyl group donor is myristoyl-acyl carrier protein. Under the conditions employed, the Escherichia **coli** precursor, Kdo(2)-lipid IVA, functions as a myristate acceptor. Introduction of the **Haemophilus htrB** gene into an E. **coli mutant** lacking **htrB** complements the biochemical and physiological defects associated with the E. **coli htrB mutation.**

Tumor necrosis factor alpha (TNF alpha) assays using murine and human macrophage cells indicated that nontypeable H. influenzae (NtHi) strain 2019 and H. influenzae type b strain A2 elicit levels of expression of TNF alpha that are 30-40 times greater than levels induced by the isogenic **htrB mutants** (B29 and A2B29). Studies using cell-free LOS indicated that the LOS from wild type strain 2019 elicits levels of TNF alpha expression that are 6-8-fold higher than those of B29. In situ hybridization studies of a primary human bronchial epithelial cell line demonstrated a greater increase of TNF alpha message produced in the presence of 2019 LOS than in the presence of B29 LOS. TNF alpha levels of the cell supernatant of cells stimulated with 2019 LOS were found to be 7-8-fold higher than levels in B29 stimulated supernatants. Using the Limulus amoebocyte lysate for assessment of endotoxic activity, we found that wild type LOS was 8-fold higher in endotoxic activity compared with the **mutant** LOS. In virulence assays using intraperitoneal inoculation of infant rats, the **htrB** isogenic strain caused bacteremia at 50% the frequency of the wild type strain. In intranasal inoculation studies, the **htrB mutant** strain was unable to cause bacteremia whereas the wild type b parent produced bacteremia in 40-60% of the animals. These findings suggest that the **htrB** gene of H. influenzae is important for virulence and that host TNF alpha expression is attenuated in response to **htrB mutant** strains.
ISSN: 0968-0519

08773215 GENUINE ARTICLE#: XT850 NUMBER OF REFERENCES: 69

TITLE: Identification of the gene encoding the Escherichia **coli** lipid a 4'-kinase - Facile phosphorylation of endotoxin analogs with recombinant LpxK

AUTHOR(S): Garrett TA; Kadrmas JL; Raetz CRH (REPRINT)
CORPORATE SOURCE: DUKE UNIV,MED CTR, DEPT BIOCHEM/DURHAM//NC/27710 (REPRINT); DUKE UNIV,MED CTR, DEPT BIOCHEM/DURHAM//NC/27710

PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N35 (AUG 29), P 21855-21864
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
ISSN: 0021-9258
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The genes for seven of nine enzymes needed for the biosynthesis of Kdo(2)-lipid A (Re endotoxin) in Escherichia **coli** have been reported, We have now identified a novel gene encoding the lipid A 4'-kinase (the sixth step of the pathway). The 4'-kinase transfers the (gamma) over dot-phosphate of ATP to the 4'-position of a tetraacyldisaccharide 1-phosphate intermediate (termed DS-1-P) to form tetraacyldisaccharide 1,4'-bis-phosphate (lipid IVA). The 4'-phosphate is required for the action of distal enzymes, such as Kdo transferase and also renders lipid A substructures active as endotoxin antagonists or mimetics, Lysates of E. **coli** generated using individual A clones from the ordered Kohara library were assayed for over-production of 4'-kinase. Only one clone, [218]E1D1, which directed 2-2.5-fold overproduction, was identified. This construct contains 20 kilobase pairs of E. **coli** DNA from the vicinity of minute 21. Two genes related to the lipid A system map in this region: msbA, encoding a putative translocator, and kdsB, the structural gene for CMP-Kdo synthase, msbA forms an operon with a downstream, essential open reading frame of unknown function, designated orfE. orfE was cloned into a T7 expression system. Washed membranes from cells overexpressing orfE display similar to 2000-fold higher specific activity of 4'-kinase than membranes from cells with vector alone. Membranes containing recombinant, overexpressed 4'-kinase (but not membranes with wild-type kinase levels) efficiently phosphorylate three DS-1-P analogs: 3-aza-DS-1-P, base-treated DS-1-P, and base-treated 3-aza-DS-1-P. A synthetic hexaacylated DS-1-P analog, compound 505, can also be phosphorylated by membranes from the overproducer, yielding [4'-P-32] lipid A (endotoxin). The overexpressed lipid A 4'-kinase is very useful for making new 4'-phosphorylated lipid A analogs with potential utility as endotoxin mimetics or antagonists, We suggest that orfE is the structural gene for the 4'-kinase and that it be redesignated IpxK.

ISSN: 0021-9258

08764778 GENUINE ARTICLE#: XT772 NUMBER OF REFERENCES: 36
TITLE: **Mutation** of the **htrB** gene in a virulent **Salmonella** typhimurium strain by intergeneric transduction: Strain construction and phenotypic characterization
AUTHOR(S): Sunshine MG; Gibson BW; Engstrom JJ; Nichols WA; Jones BD; Apicella MA (REPRINT)
CORPORATE SOURCE: UNIV IOWA,COLL MED, DEPT MICROBIOL, BSB 3-403, 51 NEWTON RD/IOWA CITY//IA/52242 (REPRINT); UNIV IOWA,COLL MED, DEPT MICROBIOL/IOWA CITY//IA/52242; UNIV CALIF SAN FRANCISCO,SCH PHARM, DEPT PHARMACEUT CHEM/SAN FRANCISCO//CA/94143
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1997, V179, N17 (SEP), P5521-5533
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0021-9193
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The **htrB** gene product of **Haemophilus** influenzae contributes to the toxicity of the lipooligosaccharide. The **htrB** gene encodes a 2-keto-3-deoxyoctulosonic acid-dependent acyltransferase which is responsible for myristic acid substitutions at the hydroxy moiety of lipid A beta-hydroxymyristic acid. Mass spectroscopic analysis has demonstrated that lipid A from an H. influenzae **htrB mutant** is predominantly tetraacyl and similar in structure to lipid TV,, which has been shown to be nontoxic in animal models. We sought to construct a **Salmonella** typhimurium **htrB mutant** in order to investigate the contribution of **htrB** to virulence in a well-defined murine typhoid model of animal pathogenesis. To this end, all r(-) m(+) galE mutS recD strain of S. typhimurium was constructed (MGS-7) and used in inter-and intrastrain transduction experiments with both coliphage P1 and **Salmonella** phage P22. The Escherichia **coli htrB** gene containing a mini-Tn10 insertion was transduced from E. call MLK217 into S. typhimurium MGS-7 via phage P1 and subsequently via phage P22 into the virulent-**Salmonella** strain SL1344. All S. typhimurium transductants showed phenotypes similar to those described for the E. coil **htrB mutant**. Mass spectrometric analysis of the crude lipid A fraction from the lipopolysaccharide of the S. typhimurium **htrB mutant** strain showed that for the dominant hexaacyl form, a lauric acid moiety was lost at one position on the lipid A and a palmitic acid moiety,vas added at another position; for the less abundant heptaacyl species, the lauric acid was rc:placed with palmitoleic acid.
ISSN: 0021-9193

3/3,AB/10 (Item 10 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

Searcher : Shears 308-4994

08467502 GENUINE ARTICLE#: WZ944 NUMBER OF REFERENCES: 36
TITLE: The outer membrane of lipid A-deficient Escherichia **coli** **mutant** LH530 has reduced levels of OmpF and leaks periplasmic enzymes
AUTHOR(S): Nurminen M (REPRINT); Hirvas L; Vaara M
CORPORATE SOURCE: UNIV HELSINKI,DEPT BACTERIOL & IMMUNOL, HAARTMAN INST, HAARTMANINKATU 3, POB 21/SF-00014 HELSINKI//FINLAND/ (REPRINT)
PUBLICATION TYPE: JOURNAL
PUBLICATION: MICROBIOLOGY-UK, 1997, V143, ,5 (MAY), P1533-1537
PUBLISHER: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING, BERKS, ENGLAND RG7 1AE
ISSN: 1350-0872
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: We have previously described a new Escherichia **coli** K-12 **mutant**, LH530, which has a defective outer membrane. LH530 is very sensitive to hydrophobic antibiotics, does not grow at 42 degrees C and synthesizes reduced amounts of lipid A. Phenotypically LH530 is very similar to the known lipid A biosynthesis **mutants** of E. **coli** and **Salmonella** typhimurium. Its genetic defect is not known, but the defect is suppressed by multiple copies of ORF195. Here we show that at 37 degrees C LH530 contains a reduced amount of the OmpF porin and that it leaks periplasmic beta-lactamase at 37 degrees C and 42 degrees C. We further show that ORF195, when present at low copy number, restores the antibiotic resistance and lipid A biosynthesis of LH530 at 28 degrees C. but not at higher temperatures. In contrast, OmpF expression is restored at 37 degrees C.
ISSN: 1350-0872

3/3,AB/11 (Item 11 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08382629 GENUINE ARTICLE#: WV262 NUMBER OF REFERENCES: 34
TITLE: Function of the Escherichia **coli** msbB gene, a multicopy suppressor of **htrB** knockouts, in the acylation of lipid A - Acylation by MsbB follows laurate incorporation by **HtrB**
AUTHOR(S): Clementz T; Zhou ZM; Raetz CRH (REPRINT)
CORPORATE SOURCE: DUKE UNIV,MED CTR, DEPT BIOCHEM/DURHAM//NC/27710 (REPRINT); DUKE UNIV,MED CTR, DEPT BIOCHEM/DURHAM//NC/27710
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N16 (APR 18), P 10353-10360
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
ISSN: 0021-9258
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Overexpression of the Escherichia **coli** msbB gene on high

copy plasmids suppresses the temperature-sensitive growth associated with **mutations** in the **htrB** gene, **htrB** encodes the lauroyl transferase of lipid A biosynthesis that acylates the intermediate (Kdo)(2)-lipid IVA (Brozek, It. A. and Raetz, C. R. H. (1990) J. Biol. Chem, 265, 15410-15417). Since msbB displays 27.5% identity and 42.2% similarity to **htrB**, me explored the possibility that msbB encodes a related acyltransferase, In contrast to **htrB**, extracts of strains with insertion **mutations** in msbB are not defective in transferring laurate from lauroyl acyl carrier protein to (Kdo)(2)-lipid IVA. However, extracts of msbB **mutants** do not efficiently acylate the product formed by **HtrB**, designated (IZdo)(2)-(lauroyl)-lipid IVA. Extracts of strains harboring msbB(+) bearing plasmids acylate (Kdo)(2)-(lauroyl)-lipid IVA very rapidly compared with wild type, We solubilized and partially purified MsbB from an overproducing strain, lacking **HtrB**, MsbB transfers myristate or laurate, activated on ACP, to (Kdo)(2)-(lauroyl)-lipid IVA. Decanoyl, palmitoyl, palmitoleoyl, and (R)-3-hydroxymyristoyl-ACP are poor acyl donors, MsbB acylates (Kdo)(2)-(lauroyl)-lipid IVA about 100 times faster than (Kdo)(2)-lipid IVA. The slow, but measurable, rate whereby MsbB acts on (Kdo)(2)-lipid IVA may explain why overexpression of MsbB suppresses the temperature-sensitive phenotype of **htrB mutations**, Presumably, the acyloxyacyl group generated by excess MsbB substitutes for the one normally formed by **HtrB**.

ISSN: 0021-9258

3/3,AB/12 (Item 12 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08131494 GENUINE ARTICLE#: WE411 NUMBER OF REFERENCES: 52
TITLE: The lipid A biosynthesis deficiency of the Escherichia **coli** antibiotic-supersensitive **mutant** LH530 is suppressed by a novel locus, ORF195
AUTHOR(S): Hirvas L (REPRINT); Nurminen M; Helander IM; Vuorio R; Vaara M
CORPORATE SOURCE: UNIV HELSINKI,HAARTMAN INST, DEPT BACTERIOL & IMMUNOL, POB 21, HAARTMANINKATU 3/SF-00014 HELSINKI//FINLAND/ (REPRINT); NATL PUBL HLTH INST,DEPT BACTERIAL VACCINE RES & MOL BIOL/FIN-00300 HELSINKI//FINLAND/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MICROBIOLOGY-UK, 1997, V143, ,1 (JAN), P73-81
PUBLISHER: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING, BERKS, ENGLAND RG7 1AE
ISSN: 1350-0872
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: A new **mutant** of Escherichia **coli** K-12 supersensitive to both hydrophobic and large hydrophilic antibiotics was isolated and characterized. The **mutant** grew well at 28 degrees C, poorly at 37

degrees C. and did not grow at 42 degrees C. The rate of its lipid A biosynthesis was reduced as compared to that of the parent strain. This deficiency was rescued by a novel locus, ORF195, the function of which has not been elucidated. ORF195 is located in the 76 min region in the E. **coli** chromosome and encodes a hypothetical 21 <bulet>8 kDa protein with no signal sequence. ORF195 isolated from the **mutant** strain had an identical sequence to the wild-type allele, indicating a suppressor function of the gene product.
ISSN: 1350-0872

3/3,AB/13 (Item 13 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07517367 GENUINE ARTICLE#: UU880 NUMBER OF REFERENCES: 43
TITLE: **MUTATIONAL** ANALYSIS AND PROPERTIES OF THE MSBA GENE OF ESCHERICHIA **COLI**, CODING FOR AN ESSENTIAL ABC FAMILY TRANSPORTER
AUTHOR(S): POLISSI A; GEORGOPOULOS C
CORPORATE SOURCE: GLAXO RIC,DIV MICROBIOL,VIA FLEMING 2/I-37100 VERONA//ITALY/ (Reprint); CTR MED UNIV GENEVA,DEPT BIOCHIM MED/CH-1211 GENEVA 4//SWITZERLAND/
PUBLICATION: MOLECULAR MICROBIOLOGY, 1996, V20, N6 (JUN), P1221-1233
ISSN: 0950-382X
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: The **htrB** gene was discovered because its insertional inactivation interfered with Escherichia **coli** growth and viability at temperatures above 32.5 degrees C, as a result of accumulation of phospholipids, The msbA gene was originally discovered because when cloned on a low-copy-number plasmid vector it was able to suppress the temperature-sensitive growth phenotype of an **htrB** null **mutant** as well as the accumulation of phospholiplds, The msbA gene product belongs to the superfamily of ABC transporters, a universally conserved family of proteins characterized by a highly conserved ATP-binding domain, The msbA gene is essential for bacterial viability at all temperatures, In order to understand the physiological role of the MsbA protein, we **mutated** the ATP-binding domain using random PCR **mutagenesis**, Six independent **mutants** were isolated and characterized, Four of these **mutations** resulted in single-amino-acid substitutions in non-conserved residues and were able to support cell growth at 30 degrees C but not at 43 degrees C, The remaining two **mutations** behaved as recessive lethals, and resulted in single-amino-acid substitutions in Walker motif B, one of the two highly conserved regions of the ATP-binding domain, Despite the fact that neither of these two **mutant** proteins can support E, coil growth, they both retained the ability to bind ATP in vitro, In addition, we present evidence to show that N-acetyl [H-3]-glucosamine, a precursor of lipopolysaccharides, accumulates at the non-permissive temperature in the inner membrane of either **htrB** null or msbA

conditional lethal strains, Translocation of the precursor to the outer membrane is restored by transformation with a plasmid containing the wild-type msbA gene, A possible role for MsbA as a translocator of lipopolysaccharides or its precursors is discussed.
ISSN: 0950-382X

3/3,AB/14 (Item 14 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07378476 GENUINE ARTICLE#: UL250 NUMBER OF REFERENCES: 56
TITLE: FUNCTION OF THE **HTRB** HIGH TEMPERATURE REQUIREMENT GENE OF ESCHERICHIA **COLI** IN THE ACYLATION OF LIPID A - **HTRB** CATALYZED INCORPORATION OF LAURATE
AUTHOR(S): CLEMENTZ T; BEDNARSKI JJ; RAETZ CRH (Reprint)
CORPORATE SOURCE: DUKE UNIV,MED CTR,DEPT BIOCHEM/DURHAM//NC/27710 (Reprint) ; DUKE UNIV,MED CTR,DEPT BIOCHEM/DURHAM//NC/27710
PUBLICATION: JOURNAL OF BIOLOGICAL CHEMISTRY, 1996, V271, N20 (MAY 17), P 12095-12102
ISSN: 0021-9258
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: By assaying lysates of Escherichia **coli** generated with the hybrid lambda bacteriophages of an ordered library (Kohara, Y., Akiyama, K., and Isono, K. (1987) Cell 50, 495-508), we identified two clones (lambda 232 and lambda 233) capable of overexpressing the lauroyl transferase that functions after 3-deoxy-D-manno-octulosonic acid (Kdo) addition in lipid A biosynthesis (Brozek, K. A., and Raetz, C. R. H. (1990) J. Biol. Chem. 265, 15410-15417). The E. **coli** DNA inserts in lambda 232 and lambda 233 suggested that a known gene (**htrB**) required for rapid growth above 33 degrees C might encode the lauroyl transferase. Using the intermediate (Kdo)(2)-lipid IVA as the laurate acceptor, extracts of strains with transposon insertions in **htrB** were found to contain no lauroyl transferase activity. Cells harboring hybrid **htrB**(+) plasmids overproduced transferase activity 100-200-fold. The overproduced transferase was solubilized with a non-ionic detergent and purified further by DEAE-Sepharose chromatography. With lauroyl acyl carrier protein as the donor, the purified enzyme rapidly incorporated one laurate residue into (Kdo)(2)-lipid IVA. The rate of laurate incorporation was reduced by several orders of magnitude when either one or both Kdos were absent in the acceptor. With a matched set of acyl-acyl carrier proteins, the enzyme incorporated laurate 3-8 times faster than decanoate or myristate, respectively. Transfer of palmitate, palmitoleate, or R-3-hydroxymyristate was very slow. Taken together with previous studies, our findings indicate that **htrB** encodes a key, late functioning acyltransferase of lipid A biosynthesis.
ISSN: 0021-9258

3/3,AB/15 (Item 15 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06897494 GENUINE ARTICLE#: TE583 NUMBER OF REFERENCES: 30
TITLE: **MUTATION** OF THE **HTRB** LOCUS OF **HAEMOPHILUS** INFLUENZAE NONTYPABLE STRAIN 2019 IS ASSOCIATED WITH MODIFICATIONS OF LIPID A AND PHOSPHORYLATION OF THE LIPO-OLIGOSACCHARIDE
AUTHOR(S): LEE NG; SUNSHINE MG; ENGSTROM JJ; GIBSON BW; APICELLA MA (Reprint)
CORPORATE SOURCE: UNIV IOWA,DEPT MICROBIOL,BOWEN SCI BLDG,51 NEWTON RD/IOWA CITY//IA/52242 (Reprint); UNIV IOWA,DEPT MICROBIOL/IOWA CITY//IA/52242; UNIV CALIF SAN FRANCISCO,SCH PHARM,DEPT PHARMACEUT CHEM/SAN FRANCISCO//CA/94143
PUBLICATION: JOURNAL OF BIOLOGICAL CHEMISTRY, 1995, V270, N45 (NOV 10), P 27151-27159
ISSN: 0021-9258
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: The **HtrB** protein was first identified in Escherichia coil as a protein required for cell viability at high temperature, but its expression was not regulated by temperature. We isolated an **htrB** homologue from nontypable **Haemophilus** influenzae strain (NTHi) 2019, which was able to functionally complement the E, **coli htrB mutation**, The promoter for the NTHi 2019 **htrB** gene overlaps the promoter for the rfaE gene, and the two genes are divergently transcribed. The deduced amino acid sequence of NTHi 2019 **HtrB** had 56% homology to E, coil **HtrB**, In vitro transcription-translation analysis confirmed production of a protein with an apparent molecular mass of 32-33 kDa, Primer extension analysis revealed that **htrB** was transcribed from a sigma(70)-dependent consensus promoter and its expression was not affected by temperature, The expression of **htrB** and rfaE was 2.5-4 times higher in the NTHi **htrB mutant** B29 than in the parental strain, In order to study the function of the **HtrB** protein in **Haemophilus**, we generated two isogenic **htrB mutants** by shuttle **mutagenesis** using a mini-Tn3, The **htrB mutants** initially showed temperature sensitivity, but they lost the sensitivity after a few passages at 30 degrees C and were able to grow at 37 degrees C, They also showed hypersensitivity to deoxycholate and kanamycin, which persisted on passage, SDS-polyacrylamide gel electrophoresis analysis revealed that the lipo-oligosaccharide (LOS) isolated from these **mutants** migrated faster than the wild type LDS and its color changed from black to brown as has been described for E. coil **htrB mutants**. Immunoblotting analysis also showed that the LOS from the **htrB mutants** lost reactivity to a monoclonal antibody, 6E4, which binds to the wild type NTHi 2019 LOS. Electrospray ionization-mass spectrometry analysis of the O-deacylated LOS oligosaccharide indicated a modification of the core structure

characterized in part by a net loss in phosphoethanolamine. Mass spectrometric analysis of the lipid A of the **htrB mutant** indicated a loss of one or both myristic acid substitutions. These data suggest that **HtrB** is a multifunctional protein and may play a controlling role in regulating cell responses to various environmental changes.

ISSN: 0021-9258

3/3,AB/16 (Item 16 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06192373 GENUINE ARTICLE#: QJ524 NUMBER OF REFERENCES: 36
TITLE: MOLECULAR CLONING AND CHARACTERIZATION OF THE NONTYPEABLE **HAEMOPHILUS** INFLUENZAE 2019 RFAE GENE REQUIRED FOR LIPOPOLYSACCHARIDE BIOSYNTHESIS
AUTHOR(S): LEE NG; SUNSHINE MG; APICELLA MA (Reprint)
CORPORATE SOURCE: UNIV IOWA,DEPT MICROBIOL,BOWEN SCI BLDG 3-401,51 NEWTON RD/IOWA CITY//IA/52242 (Reprint); UNIV IOWA,DEPT MICROBIOL/IOWA CITY//IA/52242
PUBLICATION: INFECTION AND IMMUNITY, 1995, V63, N3 (MAR), P818-824
ISSN: 0019-9567
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: The lipooligosaccharide (LOS) of nontypeable **Haemophilus** influenzae (NTHi) is an important factor in pathogenesis and virulence. In an attempt to elucidate the genes involved in LOS biosynthesis, we have cloned the rfaE gene from NTHi 2019 by complementing a **Salmonella** typhimurium rfaE **mutant** strain with an NTHi 2019 plasmid library. The rfaE **mutant** synthesizes lipopolysaccharide (LPS) lacking heptose, and the rfaE gene is postulated to be involved in ADP-heptose synthesis. Retransformation with the plasmid containing 4 kb of NTHi DNA isolated from a reconstituted **mutant** into rfaE **mutants** gave wild-type LPS phenotypes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis confirmed the conversion of the rfaE **mutant** LPS to a wild-type LPS phenotype. Sequence analysis of a 2.4-kb Bg/II fragment revealed two open reading frames. One open reading frame encodes the RfaE protein with a molecular weight of 37.6 kDa, which was confirmed by in vitro transcription and translation, and the other encodes a polypeptide highly homologous to the Escherichia **coli HtrB** protein.
These two genes are transcribed from the same promoter region into opposite directions. Primer extension analysis of the rfaE gene revealed a single transcription start site at 37 bp upstream of the predicted translation start. site. The upstream promoter region contained a sequence (TA AAAT) homologous to the -10 region of the bacterial sigma(70)-dependent promoters at an appropriate distance (7 bp), but no sequence resembling the consensus sequence of the -35 region was found. These studies demonstrate the ability to use

complementation of defined LPS defects in members of the family Enterobacteriaceae to identify LOS synthesis genes in NTHi.
ISSN: 0019-9567

3/3,AB/17 (Item 17 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06026225 GENUINE ARTICLE#: PY759 NUMBER OF REFERENCES: 44
TITLE: SERUM-SENSITIVE **MUTATION** OF FRANCISELLA NOVICIDA - ASSOCIATION WITH AN ABC TRANSPORTER GENE
AUTHOR(S): MDLULI KE; ANTHONY LSD; BARON GS; MCDONALD MK; MYLTSEVA SV; NANO FE
CORPORATE SOURCE: UNIV VICTORIA,DEPT BIOCHEM & MICROBIOL/VICTORIA/BC V8W 3P6/CANADA/ (Reprint)
PUBLICATION: MICROBIOLOGY-UK, 1994, V140, DEC (DEC), P3309-3318
ISSN: 1350-0872
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: Francisella novicida is a facultative intracellular pathogen that can survive and grow in macrophages by preventing phagolysosomal fusion. In this study in vitro cassette **mutagenesis** was used to generate a library of insertion **mutants** of F. novicida. Two related **mutants**, KM14 and KM14S, initially identified as defective for growth in macrophages, were found to be sensitive to serum. These **mutants** were also found to grow approximately 1000-fold less well in the livers and spleens of infected mice. We cloned a genetic locus that was presumably **mutagenized** in these **mutants** and found that it included genes that had high similarity in their deduced amino acid sequence to those of msbA and orFE of Escherichia coil. The former is a member of the superfamily of ABC transporter proteins. We named the corresponding genes in F. novicida, valAB. Integration of a cloned valAB locus into the chromosome of KM14S partially restored the serum resistance phenotype found in wild-type F. novicida.
ISSN: 1350-0872

3/3,AB/18 (Item 18 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

04227251 GENUINE ARTICLE#: KE353 NUMBER OF REFERENCES: 40
TITLE: THE ESSENTIAL ESCHERICHIA-**COLI** MSBA GENE, A MULTICOPY SUPPRESSOR OF NULL **MUTATIONS** IN THE **HTRB** GENE, IS RELATED TO THE UNIVERSALLY CONSERVED FAMILY OF ATP-DEPENDENT TRANSLOCATORS
AUTHOR(S): KAROW M; GEORGOPOULOS C
CORPORATE SOURCE: TEMPLE UNIV,HLTH SCI CTR,DEPT MICROBIOL & IMMUNOL,3400 N BROAD ST/PHILADELPHIA//PA/19140 (Reprint); UNIV UTAH,SCH MED,DEPT

CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132; CTR MED UNIV GENEVA/CH-1211 GENEVA 4//SWITZERLAND/
PUBLICATION: MOLECULAR MICROBIOLOGY, 1993, V7, N1 (JAN), P69-79
ISSN: 0950-382X
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: We report the characterization of the msbA gene, isolated as a multicopy suppressor of the **HtrB** temperature-sensitive phenotype. The msbA gene maps to 20.5 min on the Escherichia **coli** genetic map and encodes a protein with an estimated molecular mass of 64460 Da, with the properties of an integral membrane protein. The amino acid sequence of MsbA is very similar to those of the family of ATP-dependent translocators, which includes the haemolysin B protein of E. **coli** and the mammalian multidrug resistance (MDR) proteins. **Mutational** analysis of msbA indicates that it may form an operon with a downstream gene, orfE, and that both of these genes are essential for bacterial viability under all growth conditions tested.
ISSN: 0950-382X

3/3,AB/19 (Item 19 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

04134812 GENUINE ARTICLE#: JY560 NUMBER OF REFERENCES: 47
TITLE: THE LETHAL PHENOTYPE CAUSED BY NULL **MUTATIONS** IN THE ESCHERICHIA-**COLI**-**HTRB** GENE IS SUPPRESSED BY **MUTATIONS** IN THE ACCBC OPERON, ENCODING 2 SUBUNITS OF ACETYL COENZYME A CARBOXYLASE
AUTHOR(S): KAROW M; FAYET O; GEORGOPOULOS C
CORPORATE SOURCE: TEMPLE UNIV,HLTH SCI CTR,DEPT MICROBIOL & IMMUNOL/PHILADELPHIA//PA/19140 (Reprint); UNIV UTAH,SCH MED,DEPT CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132; CTR RECH BIOCHIM & GENET CELLULAIRES,CNRS/F-31062 TOULOUSE//FRANCE/; UNIV GENEVA,CTR MED/CH-1211 GENEVA 4//SWITZERLAND/
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1992, V174, N22 (NOV), P7407-7418
ISSN: 0021-9193
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: Insertion **mutations** in the Escherichia **coli** **htrB** gene result in the unique phenotype of not affecting growth at temperatures below 32.5-degrees-C but leading to a loss of viability at temperatures above this in rich media. When **htrB** bacteria growing in rich media were shifted to the nonpermissive temperature of 42-degrees-C, they continued to grow at a rate similar to that at 30-degrees-C but they produced phospholipids at the rate required for growth at 42-degrees-C. This led to the accumulation of more than twice as much phospholipid per milligram of protein compared with that in wild-type bacteria. Consistent with **HtrB** playing a role in phospholipid biosynthesis, one complementation group of spontaneously arising **mutations** that suppressed **htrB**-induced lethality

were mapped to the accBC operon. This operon codes for the biotin carboxyl carrier protein and biotin carboxylase subunits of the acetyl coenzyme A carboxylase enzyme complex, which catalyzes the first step in fatty acid biosynthesis. Four suppressor **mutations** mapped to this operon. Two alleles were identified as **mutations** in the accC gene, the third allele was identified as a **mutation** in the accB gene, and the fourth allele was shown to be an insertion of an IS1 transposable element in the promoter region of the operon, resulting in reduced transcription. The suppressor **mutations** caused a decrease in the rate of phospholipid biosynthesis, restoring the balance between the biosynthesis of phospholipids and growth rate, thus enabling **htrB** bacteria to grow at high temperatures.
ISSN: 0021-9193

3/3,AB/20 (Item 20 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

03470266 GENUINE ARTICLE#: HE459 NUMBER OF REFERENCES: 38
TITLE: ISOLATION AND CHARACTERIZATION OF THE ESCHERICHIA-**COLI** MSBB GENE, A MULTICOPY SUPPRESSOR OF NULL **MUTATIONS** IN THE HIGH-TEMPERATURE REQUIREMENT GENE **HTRB**
AUTHOR(S): KAROW M; GEORGOPOULOS C
CORPORATE SOURCE: UNIV UTAH,SCH MED,DEPT CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132 (Reprint)
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1992, V174, N3 (FEB), P702-710
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: Previous work established that the **htrB** gene of Escherichia **coli** is required for growth in rich media at temperatures above 32.5-degrees-C but not at lower temperatures. In an effort to determine the functional role of the **htrB** gene product, we have isolated a multicopy suppressor of **htrB**, called msbB. The msbB gene has been mapped to 40.5 min on the E. **coli** genetic map, in a 12- to 15-kb gap of the genomic library made by Kohara et al. (Y. Kohara, K. Akiyama, and K. Isono, Cell 50:495-508, 1987). Mapping data show that the order of genes in the region is eda-edd-zwf-pykA-msbB. The msbB gene codes for a protein of 37,410 Da whose amino acid sequences is similar to that of **HtrB** and, like **HtrB**, the protein is very basic in nature. The similarity of the **HtrB** and MsbB proteins could indicate that they play functionally similar roles. **Mutational** analysis of msbB shows that the gene is not essential for E. **coli** growth; however, the **htrB** msbB double **mutant** exhibits a unique morphological phenotype at 30-degrees-C not seen with either of the single **mutants**. Analysis of both msbB and **htrB mutants** shows that these bacteria are resistant to four times more deoxycholate than wild-type bacteria but not to other hydrophobic substances. The addition of quaternary ammonium compounds rescues the temperature-sensitive phenotype of

htrB bacteria, and this rescue is abolished by the simultaneous addition of Mg2+ or Ca2+. These results suggest that MsbB and **HtrB** play an important role in outer membrane structure and/or function.

3/3,AB/21 (Item 21 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

03122489 GENUINE ARTICLE#: GG051 NUMBER OF REFERENCES: 33
TITLE: SEQUENCING, **MUTATIONAL** ANALYSIS, AND TRANSCRIPTIONAL REGULATION OF THE ESCHERICHIA-**COLI HTRB** GENE
AUTHOR(S): KAROW M; GEORGOPOULOS C
CORPORATE SOURCE: UNIV UTAH,SCH MED,DEPT CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132 (Reprint)
PUBLICATION: MOLECULAR MICROBIOLOGY, 1991, V5, N9 (SEP), P2285-2292
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: The Escherichia **coli htrB** gene was originally discovered because its insertional inactivation led to an exquisitely temperature-sensitive phenotype in rich media, i.e. the ability to form colonies at temperatures below 32-degrees-C, but not above 33-degrees-C. The **htrB** gene has been sequenced. It can potentially code for two proteins, with M(r) values of 35407 Da and 8669 Da, that are encoded by overlapping, divergent open reading frames. Our data are consistent with the 35407 Da protein being **HtrB**. Northern blot analysis clearly shows that the monocistronic **htrB** message is not under heat-shock regulation. We have also sequenced the flanking DNA and have discovered a new gene, designated orf39.9, located immediately adjacent to **htrB**, but divergently transcribed.

3/3,AB/22 (Item 22 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

02822742 GENUINE ARTICLE#: FM035 NUMBER OF REFERENCES: 30
TITLE: COMPLEX PHENOTYPES OF NULL **MUTATIONS** IN THE HTR GENES, WHOSE PRODUCTS ARE ESSENTIAL FOR ESCHERICHIA-**COLI** GROWTH AT ELEVATED TEMPERATURES
AUTHOR(S): KAROW M; RAINA S; GEORGOPOULOS C (Reprint); FAYET O
CORPORATE SOURCE: UNIV UTAH,MED CTR,DEPT CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132 (Reprint); UNIV UTAH,MED CTR,DEPT CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132; CNRS,CTR RECH BIOCHIM & GENET CELLULAIRES/F-31062 TOULOUSE//FRANCE/
PUBLICATION: RESEARCH IN MICROBIOLOGY, 1991, V142, N2-3 (FEB-APR), P289-294
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: Transposon insertion, followed by screening, has allowed the

identification of a set of genes, called htr, whose products are required for Escherichia **coli** growth at elevated temperatures. The **htrB** gene has been shown to map at 23.5 min on the E. **coli** genetic map. It codes for a very basic, hydrophobic, 35,000-Mr polypeptide, possessing a putative membrane-spanning domain. At the non-permissive temperature, **htrB mutant** bacteria stop dividing, followed by the formation of bulges and eventual lysis. The htrC gene maps at 90 min, is under sigma-32 regulation and codes for a 21, 130-Mr polypeptide. At 43-degrees-C, htrC **mutant** bacteria gradually lyse, whereas at intermediate temperatures they filament extensively. Finally, the htrM gene maps at 81 min, is under sigma-32 regulation and codes for a 35,000-Mr polypeptide. The HtrM null phenotype included inability to grow above 42-degrees-C, extreme mucoidness and sensitivity to bile salts, even at the permissive temperatures. The htrM gene is identical to the rfaD gene, whose product is required for the biosynthesis of the lipopolysaccharide precursor ADP-L-glycero-D-mannoheptose (Pegues et al., J. Bact., 1990, 172, 4652-4660).

3/3,AB/23 (Item 23 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

02530639 GENUINE ARTICLE#: ET446 NUMBER OF REFERENCES: 62
TITLE: ISOLATION AND CHARACTERIZATION OF THE ESCHERICHIA-**COLI** **HTRB** GENE, WHOSE PRODUCT IS ESSENTIAL FOR BACTERIAL VIABILITY ABOVE 33-DEGREES-C IN RICH MEDIA
AUTHOR(S): KAROW M; FAYET O; CEGIELSKA A; ZIEGELHOFFER T; GEORGOPOULOS C
CORPORATE SOURCE: UNIV UTAH,SCH MED,DEPT CELLULAR VIRAL & MOLEC BIOL/SALT LAKE CITY//UT/84132 (Reprint); CNRS,CTR RECH BIOCHIM & GENET CELLULAIRES/F-31062 TOULOUSE//FRANCE/
PUBLICATION: JOURNAL OF BACTERIOLOGY, 1991, V173, N2 (JAN), P741-750
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: We have identified and studied the **htrB** gene of Escherichia **coli**. Insertional inactivation of the **htrB** gene leads to bacterial death at temperatures above 33-degrees-C. The **mutant** bacterial phenotype at nonpermissive temperatures includes an arrest of cell division followed by the formation of bulges or filaments. The **htrB+** gene has been cloned by complementation and shown to reside at 23.4 min on the E. **coli** genetic map, the relative order of the neighboring loci being mboA-**htrB**-pyrC. The **htrB** gene is transcribed in a counterclockwise fashion, relative to the E. **coli** genetic map, and its product has been identified as a membrane-associated protein of 35,000 Da. Growth experiments in minimal media indicate that the **HtrB** function becomes dispensable at low growth rates.

3/3,AB/24 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

11999709 PASCAL No.: 95-0187031
Molecular cloning and characterization of the nontypeable **Haemophilus** influenzae 2019 rfaE gene required for lipopolysaccharide biosynthesis
NA-GYONG LEE; SUNSHINE M G; APICELLA M A
Univ. Iowa, dep. microbiology, Iowa City IA 52242, USA
Journal: Infection and immunity, 1995, 63 (3) 818-824
Language: English
The lipooligosaccharide (LOS) of nontypeable **Haemophilus** influenzae (NTHi) is an important factor in pathogenesis and virulence. In an attempt to elucidate the genes involved in LOS biosynthesis, we have cloned the rfaE gene from NTHi 2019 by complementing a **Salmonella** typhimurium rfaE **mutant** strain with an NTHi 2019 plasmid library. The rfaE **mutant** synthesizes lipopolysaccharide (LPS) lacking heptose, and the rfaE gene is postulated to be involved in ADP-heptose synthesis. Retransformation with the plasmid containing 4 kb of NTHi DNA isolated from a reconstituted **mutant** into rfaE **mutants** gave wild-type LPS phenotypes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis confirmed the conversion of the rfaE **mutant** LPS to a wild-type LPS phenotype. Sequence analysis of a 2.4-kb BglII fragment revealed two open reading frames. One open reading frame encodes the RfaE protein with a molecular weight of 37.6 kDa, which was confirmed by in vitro transcription and translation, and the other encodes a polypeptide highly homologous to the Escherichia **coli HtrB** protein. These two genes are transcribed from the same promoter region into opposite directions. Primer extension analysis of the rfaE gene revealed a single transcription start site at 37 bp upstream of the predicted translation start site. The upstream promoter region contained a sequence (TA AAAT) homologous to the - 10 region of the bacterial SUP 7 SUP 0 -dependent promoters at an appropriate distance (7 bp), but no sequence resembling the consensus sequence of the -35 region was found. These studies demonstrate the ability to use complementation of defined LPS defects in members of the family Enterobacteriaceaa to identify LOS synthesis genes in NTHi

3/3,AB/25 (Item 1 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03051075 H.W. WILSON RECORD NUMBER: BGSI95051075
How **Salmonella** survive against the odds.
Foster, John W
Spector, Michael P
Annual Review of Microbiology (Annu Rev Microbiol) v. 49 ('95) p. 145-74
DOCUMENT TYPE: Feature Article

SPECIAL FEATURES: bibl il ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 14614

ABSTRACT: The enteric pathogen **Salmonella** typhimurium faces daunting odds during its voyages in the natural environment and through an infected host. It must manage stresses ranging from feast to famine, acid to base, and high to low osmolarity, among others, as well as counter various types of oxidative stress and a variety of antimicrobial peptides. The defenses used to survive these encounters can be specific or can provide cross protection to a variety of hostile conditions. Once inside a host, **Salmonella** spp. escape the extracellular environment and thus humoral immunity by invading professional and nonprofessional phagocytes in which a new set of challenges await. Some of these stresses are similar to those encountered in the natural environment (e.g. acid, starvation) but the bacterial response is complicated by the simultaneous occurrence of multiple stresses. S. typhimurium appears to sense various in vivo cues and responds by seducing the host signal-transduction pathways that are required to phagocytize the bacterial cell. The pathogen then calls upon components of its stress-response arsenal to survive the intracellular environment. These survival strategies enable the organism to persist in nature, where conditions are usually suboptimal and equip the bacterium with pathogenic properties that, if successful, will provide it with a very rich and stress-free growth environment, a dead host. Reprinted by permission of the publisher.

01252959 AAD9234745
MOLECULAR GENETICS OF THE ESCHERICHIA **COLI HTRB** GENE
Author: KAROW, MARGARET LEE
Degree: PH.D.
Year: 1992
Corporate Source/Institution: THE UNIVERSITY OF UTAH (0240)
Source: VOLUME 53/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 3313. 182 PAGES

The Escherichia **coli htrB** gene was identified during an insertional **mutagenesis** screen for new heat shock genes. **HtrB** is essential for viability in rich media only at temperatures above 32.5$\sp\circ$C, a phenotype related to bacterial growth rate, since **htrB** bacteria are viable at high temperatures in minimal medium. Despite its unique temperature-sensitive phenotype, the **htrB** gene is not under heat shock regulation. When grown at nonpermissive temperatures, **htrB** bacteria exhibit density-dependent morphological alterations,

including the formation of bulges and filaments. The lipopolysaccharide layer of the outer membrane may be altered in **htrB** bacteria, as indicated by the ability of cationic detergents to reverse the lethal phenotype and by the increased resistance of **htrB** bacteria to deoxycholate.

Four spontaneously arising **mutations** that suppress the **HtrB** temperature-sensitive phenotype were mapped to the accBC operon, encoding two of the subunits of acetyl-CoA carboxylase, which catalyzes the first step in fatty acid biosynthesis. Biochemical analysis indicates that **htrB mutant** bacteria overproduce phospholipids at nonpermissive temperatures, a phenotype closely correlated with loss in viability. The accBC **mutations** most likely suppress the lethal phenotype of **htrB** by lowering the rate of fatty acid biosynthesis, thus inhibiting the phospholipid overproduction.

Two new genes were also identified in this study as multicopy suppressors of **htrB**. The protein encoded by the msbA suppressor is related to the ATP-dependent translocator family of proteins involved in the export of molecules out of cells. The msbA gene is a unique member of this family because it is essential for bacterial viability. The orfE gene, which is coexpressed with msbA, is also essential. The protein encoded by the msbB suppressor gene appears to play a similar, if not redundant role to **HtrB**, because MsbB and **HtrB** have similar amino acid sequences and structural feature. Furthermore, **htrB** msbB double **mutant** bacteria exhibit both morphological alterations and growth defects at 30$\sp\circ$C, phenotypes that are not exhibited by either of the single **mutants**. Although bacteria with msbB null **mutations** are viable, they also exhibit an increased resistance to deoxycholate, indicating that like **HtrB**, MsbB may play a role in outer membrane function.

? ds

Set	Items	Description
S4	338	AU=(APICELLA, M? OR APICELLA M?)
S5	87	AU=(SUNSHINE, M? OR SUNSHINE M?)
S6	2431	AU=(LEE, N? OR LEE N?)
S7	28	AU=(ARUMUGHAM R? OR ARUMUGHAM, R?)
S8	931	AU=(GIBSON, B? OR GIBSON B?)
S9	0	S4 AND S5 AND S6 AND S7 AND S8
S10	74	S4 AND (S5 OR S6 OR S7 OR S8)
S11	23	S5 AND (S6 OR S7 OR S8)
S12	10	S6 AND (S7 OR S8)
S13	0	S7 AND S8
S14	3708	S4 OR S5 OR S6 OR S7 OR S8
S15	25	(S10 OR S14) AND S1
S16	33	S11 OR S12 OR S15
S18	26	S16 NOT S3

-Author (s)

Searcher : Shears 308-4994

S19 7 RD (unique items)
? t 19/3,ab/1-7

19/3,AB/1 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08310828 GENUINE ARTICLE#: WQ298 NUMBER OF REFERENCES: 26
TITLE: Identification of the ADP-L-glycero-D-manno-heptose-6-epimerase (rfaD) and heptosyltransferase II (rfaF) biosynthesis genes from nontypeable Haemophilus influenzae 2019
AUTHOR(S): Nichols WA; **Gibson BW**; Melaugh W; **Lee NG**; **Sunshine M**; Apicella MA (REPRINT)
CORPORATE SOURCE: UNIV IOWA,COLL MED, DEPT MICROBIOL, BSB 3-403, 51 NEWTON RD/IOWA CITY//IA/52242 (REPRINT); UNIV IOWA,COLL MED, DEPT MICROBIOL/IOWA CITY//IA/52242; UNIV CALIF SAN FRANCISCO,SCH PHARM, DEPT PHARMACEUT CHEM/SAN FRANCISCO//CA/94143; UNIV SAN FRANCISCO,DEPT CHEM/SAN FRANCISCO//CA/94117
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N4 (APR), P1377-1386
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Haemophilus influenzae is an important human pathogen, The lipooligosaccharide (LOS) of H. influenzae has been implicated as a virulence determinant, To better understand the assembly of LOS in nontypeable H. influenzae (NtHi), we have cloned and characterized the rfaD and rfaF genes of NtHi 2019, which encode the ADP-L-glycero-D-manno-heptose-6-epimerase and heptosyltransferase II enzymes, respectively, This cloning was accomplished by the complementation of Salmonella typhimurium lipopolysaccharide (LPS) biosynthesis gene mutants, These deep rough mutants are novobiocin susceptible until complemented with the appropriate gene, In this manner, we are able to use novobiocin resistance to select for specific NtHi LOS inner core biosynthesis genes, Such a screening system yielded a plasmid with a 4,8-kb insert, This plasmid was able to complement both rfaD and rfaF mutants of S. typhimurium. The LPS of these complemented strains appeared identical to the wild-type Salmonella LPS, The genes encoding the rfaD and rfaF genes from NtHi 2019 were sequenced and found to be similar to the analogous genes from S. typhimurium and Escherichia coli, The rfaD gene encodes a polypeptide of 35 kDa and the rfaF encodes a protein of 39 kDa, as demonstrated by in vitro transcription-translation studies. Isogenic mutants which demonstrated truncated LOS consistent with inner core biosynthesis mutants were constructed in the NtHi strain 2019, Primer extension analysis demonstrated the presence of a strong promoter upstream of rfaD but suggested only a very weak promoter upstream of rfaF,

Complementation studies, however, suggest that the rfaF gene does have an independent promoter, Mass spectrometric analysis shows that the LOS molecules expressed by H. influenzae rfaD and rfaF mutant strains have identical molecular masses. Additional studies verified that in the rfaD mutant strain, D-glycero-D-mannoheptose is added to the LOS molecule in place of the usual L-glycero-D-manno-heptose. Finally, the genetic organizations of the inner core biosynthesis genes of S. typhimurium, E. coli, and several strains of H. influenzae were examined, and substantial differences were uncovered.

ISSN: 0019-9567

19/3,AB/2 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00736156 97240526

Study of the role of the **htrB** gene in **Salmonella** typhimurium virulence

Jones B.D.; Nichols W.A.; **Gibson B.W.**; **Sunshine M.G.**; **Apicella M.A.**
ADDRESS: B.D. Jones, Department of Microbiology, Univ. of Iowa College of Medicine, Iowa City, IA 52242-1109, United States
EMAIL: bjones@blue.weeg.uiowa.edu

Journal: Infection and Immunity, 65/11 (4778-4783), 1997, United States
PUBLICATION DATE: 19970000
CODEN: INFIB
ISSN: 0019-9567
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 70

We have undertaken a study to investigate the contribution of the **htrB** gene to the virulence of pathogenic **Salmonella** typhimurium. An **htrB**::mini- Tn10 mutation from Escherichia call was transferred by transduction to the mouse-virulent strain S. typhimurium SL1344 to create an **htrB** mutant. The and typhimurium **htrB** mutant was inoculated into mice and found to be severely limited in its ability to colonize organs of the lymphatic system and to cause systemic disease in mice. A variety of experiments were performed to determine the possible reasons for this loss of virulence. Serum killing assays revealed that the S. typhimurium **htrB** mutant was as resistant to killing by complement as the wild-type strain. However, macrophage survival assays revealed that the and typhimurium **htrB** mutant was more sensitive to the intracellular environment of murine macrophages than the wild-type strain. In addition, the bioactivity of the lipopolysaccharide (LPS) of the **htrB** mutant was reduced compared to that of the LPS from the parent strain as measured by both a Limulus amoebocyte lysate endotoxin quantitation assay and a tumor necrosis factor alpha bioassay. These

results indicate that the **htrB** gene plays a role in the virulence of S. typhimurium.

19/3,AB/3 (Item 2 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00736108 97240478
Evaluation of the virulence of nontypeable **Haemophilus** influenzae lipooligosaccharide **htrB** and rfaD mutants in the chinchilla model of otitis media
DeMaria T.F.; **Apicella M.A.**; Nichols W.A.; Leake E.R.
ADDRESS: T.F. DeMaria, Division of Otologic Research, Ohio State University, College of Medicine, 456 West 10th Ave., Columbus, OH 43210, United States
EMAIL: tdemaria@pop.service.acs.ohio-state.edu
Journal: Infection and Immunity, 65/11 (4431-4435), 1997, United States
PUBLICATION DATE: 19970000
CODEN: INFIB
ISSN: 0019-9567
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 30

Considerable evidence has implicated nontypeable **Haemophilus** influenzae (NTHi) lipooligosaccharide (LOS) in the pathogenesis of otitis media (OM); however, its exact role has not been conclusively established. Recently, two NTHi LOS-deficient mutants have been created and described. Strain 2019-DK1, an rfaD gene mutant, expresses a truncated LOS consisting of only three deoxy-D-manno-octulosonic acid residues, a single heptose, and lipid A. Strain 2019-B29, an isogenic **htrB** mutant, possesses an altered oligosaccharide core and an altered lipid A. Each strain's ability to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation was evaluated in the chinchilla model. Nasopharyngeal colonization data indicate that the parent strain and both mutants are able to colonize the nasopharynx and exhibit comparable clearance kinetics. Compared with the parent and each other, however, the mutants demonstrated marked differences in virulence regarding their relative abilities to induce OM and persist in the middle ear post-transbullar inoculation. Strain B29 required a 3-log-greater dose to induce OM than the parent strain and did not exhibit evidence of sustained multiplication but persisted for the same duration as the parent. Conversely, strain-DK1, even when inoculated at a dose 4 logs greater than the parent dose, was eliminated from the middle ear 72 h after challenge. A comparison of the relative pathogenicities of these isolates provides the opportunity to address fundamental questions regarding the contribution of LOS to pathogenesis issues at the molecular level. Specifically, the impact of these LOS gene disruptions on OM pathogenesis can be defined and may thus

provide potential new targets for future protection and intervention strategies.

19/3,AB/4 (Item 3 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00699175 97202783
Mutation of the **htrB** gene in a virulent **Salmonella** typhimurium strain by intergeneric transduction: Strain construction and phenotypic characterization
Sunshine M.G.; **Gibson B.W.**; Engstrom J.J.; Nichols W.A.; Jones B.D.; **Apicella M.A.**
ADDRESS: M.A. Apicella, Department of Microbiology, Univ. of Iowa College of Medicine, 51 Newton Rd., Iowa City, IA 52242, United States
EMAIL: michael-apicella@uiowa.edu
Journal: Journal of Bacteriology, 179/17 (5521-5533), 1997, United States
PUBLICATION DATE: 19970000
CODEN: JOBAA
ISSN: 0021-9193
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 36

The **htrB** gene product of **Haemophilus** influenzae contributes to the toxicity of the lipooligosaccharide. The **htrB** gene encodes a 2-keto-3- deoxyoctulosonic acid-dependent acyltransferase which is responsible for myristic acid substitutions at the hydroxy moiety of lipid A beta- hydroxymyristic acid. Mass spectroscopic analysis has demonstrated that lipid A from an H. influenzae **htrB** mutant is predominantly tetraacyl and similar in structure to lipid IV(A), which has been shown to be nontoxic in animal models. We sought to construct a **Salmonella** typhimurium **htrB** mutant in order to investigate the contribution of **htrB** to virulence in a well-defined murine typhoid model of animal pathogenesis. To this end, an rsup - msup + gale mutS recD strain of S. typhimurium was constructed (MGS-7) and used in inter- and intrastrain transduction experiments with both coliphage P1 and **Salmonella** phage P22. The Escherichia **coli htrB** gene containing a mini-Tn10 insertion was transduced from E, **coli** MLK217 into S. typhimurium MGS-7 via phage P1 and subsequently via phage P22 into the virulent **Salmonella** strain SL1344. All S. typhimurium transductants showed phenotypes similar to those described for the E. **coli htrB** mutant. Mass spectrometric analysis of the crude lipid A fraction from the lipopolysaccharide of the S. typhimurium **htrB** mutant strain showed that for the dominant hexaacyl form, a lauric acid moiety was lost at one position on the lipid A and a palmitic acid moiety was added at another position; for the less abundant haptaacyl species, the lauric acid was replaced with palmitoleic acid.

19/3,AB/5 (Item 4 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00680306 97187075
htrB of **Haemophilus** influenzae: Determination of biochemical activity and effects on virulence and lipooligosaccharide toxicity
Nichols W.A.; Raetz C.R.H.; Clementz T.; Smith A.L.; Hanson J.A.; Ketterer M.R.; **Sunshine M.**; **Apicella M.A.**
ADDRESS: Dr. M.A. Apicella, Dept. of Microbiology ESB 3-403, Univ. of Iowa Coll. of Medicine, 51 Newton Road, Iowa City, IA 522442, United States
EMAIL: michael-apicella@uiowa.edu
Journal: Journal of Endotoxin Research, 4/3 (163-172), 1997, United Kingdom
PUBLICATION DATE: 19970000
CODEN: JENRE
ISSN: 0968-0519
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 28

The **htrB** mutant of **Haemophilus** influenzae (strain B29) has been shown to lack secondary (nonhydroxylated) acyl groups in its lipid A. We have determined through in vitro biochemical assays that the **HtrB** protein acts as a specific acyltransferase in the late stages of lipid A biosynthesis and that the preferred acyl group donor is myristoyl-acyl carrier protein. Under the conditions employed, the Escherichia coil precursor, Kdoinf 2-lipid IV(A), functions as a myristate acceptor. Introduction of the **Haemophilus htrB** gene into an E. **coli** mutant lacking **htrB** complements the biochemical and physiological defects associated with the E. **coli htrB** mutation. Tumor necrosis factor alpha (TNFalpha) assays using murine and human macrophage cells indicated that nontypeable H. influenzae (NtHi) strain 2019 and H. influenzae type b strain A2 elicit levels of expression of TNFalpha that are 30-40 times greater than levels induced by the isogenic **htrB** mutants (B29 and A2B29). Studies using cell-free LOS indicated that the LOS from wild type strain 2019 elicits levels of TNFalpha expression that are 6-8-fold higher than those of B29. In situ hybridization studies of a primary human bronchial epithelial cell line demonstrated a greater increase of TNFalpha message produced in the presence of 2019 LOS than in the presence of B29 LOS. TNFalpha levels of the cell supernatant of cells stimulated with 2019 LOS were found to be 7-8-fold higher than levels in B29 stimulated supernatants. Using the Limulus amoebocyte lysate for assessment of endotoxic activity, we found that wild type LOS was 8-fold higher in endotoxic activity compared with the mutant LOS. In virulence assays using intraperitoneal inoculation of infant rats, the **htrB** isogenic strain caused bacteremia at 50% the frequency of the wild type

strain. In intranasal inoculation studies, the **htrB** mutant strain was unable to cause bacteremia whereas the wild type b parent produced bacteremia in 40-60% of the animals. These findings suggest that the **htrB** gene of H. influenzae is important for virulence and that host TNFalpha expression is attenuated in response to **htrB** mutant strains.

19/3,AB/6 (Item 5 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00323784 96007283
Mutation of the **htrB** locus of **Haemophilus** influenzae nontypable strain 2019 is associated with modifications of lipid A and phosphorylation of the lipo-oligosaccharide
Lee N.-G.; **Sunshine M.G.**; Engstrom J.J.; **Gibson B.W.**; **Apicella M.A.**
ADDRESS: M.A. Apicella, Dept. of Microbiology, Bowen Science Bldg., University of Iowa, 51 Newton Rd., Iowa City, IA 52242, United States
Journal: Journal of Biological Chemistry, 270/45 (27151-27159), 1995, United States
PUBLICATION DATE: 19950000
CODEN: JBCHA
ISSN: 0021-9258
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English

The **HtrB** protein was first identified in Escherichia **coli** as a protein required for cell viability at high temperature, but its expression was not regulated by temperature. We isolated an **htrB** homologue from non-typable **Haemophilus** influenzae strain (NTHi) 2019, which was able to functionally complement the E. **coli htrB** mutation. The promoter for the NTHi 2019 **htrB** gene overlaps the promoter for the rfaE gene, and the two genes are divergently transcribed. The deduced amine acid sequence of NTHi 2019 **HtrB** had 56% homology to E. **coli HtrB**. In vitro transcription-translation analysis confirmed production of a protein with an apparent molecular mass of 32-33 kDa. Primer extension analysis revealed that htrE was transcribed from sigmasup 7sup 0-dependent consensus promoter and its expression was not affected by temperature. The expression of **htrB** and rfaE was 2.5-4 times higher in the NTHi **htrB** mutant B29 than in the parental strain. In order to study the function of the **HtrB** protein in **Haemophilus**, we generated two isogenic **htrB** mutants by shuttle mutagenesis using a mini-Tn3. The **htrB** mutants initially showed temperature sensitivity, but they lost the sensitivity after a few passages at 30 degreeC and were able to grow at 37 degreeC. They also showed hypersensitivity to deoxycholate and kanamycin, which persisted on passage. SDS-polyacrylamide gel electrophoresis analysis revealed that the lipo- oligosaccharide (LOS)

isolated from these mutants migrated faster than the wild type LOS and its color changed from black to brown as has been described for E. **coli htrB** mutants. Immunoblotting analysis also showed that the LOS from the **htrB** mutants lost reactivity to a monoclonal antibody, 6E4, which binds to the wild type NTHi 2019 LOS. Electrospray ionization-mass spectrometry analysis of the O-deacylated LOS oligosaccharide indicated a modification of the core structure characterized in part by a net loss in phosphoethanolamine. Mass spectrometric analysis of the lipid A of the **htrB** mutant indicated a loss of one r both myristic acid substitutions. These data suggest that **HtrB** is a multifunctional protein and may play a controlling role in regulating cell responses to various environmental changes.

19/3,AB/7 (Item 6 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00248954 95047178
Molecular cloning and characterization of the nontypeable **Haemophilus** influenzae 2019 rfaE gene required for lipopolysaccharide biosynthesis
Lee N.-G.; Sunshine M.G.; Apicella M.A.
ADDRESS: M.A. Apicella, Department of Microbiology, Bowen Science Building, University of Iowa, 3-401, 51 Newton Road, Iowa City, IA 52242, United States
Journal: Infection and Immunity, 63/3 (818-824), 1995, United States
PUBLICATION DATE: 19950000
CODEN: INFIB
ISSN: 0019-9567
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English

The lipooligosaccharide (LOS) of nontypeable **Haemophilus** influenzae (NTHi) is an important factor in pathogenesis and virulence. In an attempt to elucidate the genes involved in LOS biosynthesis, we have cloned the rfaE gene from NTHi 2019 by complementing a **Salmonella** typhimurium rfaE mutant strain with an NTHi 2019 plasmid library. The rfaE mutant synthesizes lipopolysaccharide (LPS) lacking heptose, and the rfaE gene is postulated to be involved in ADP-heptose synthesis. Retransformation with the plasmid containing 4 kb of NTHi DNA isolated from a reconstituted mutant into rfaE mutants gave wild-type LPS phenotypes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis confirmed the conversion of the rfaE mutant LPS to a wild-type LPS phenotype. Sequence analysis of a 2.4-kb BglII fragment revealed two open reading frames. One open reading frame encodes the RfaE protein with a molecular weight of 37.6 kDa, which was confirmed by in vitro transcription and translation, and the other encodes a polypeptide highly homologous to the Escherichia **coli HtrB** protein. These two genes are transcribed from the same promoter region into opposite directions. Primer extension analysis of the rfaE gene

revealed a single transcription start site at 37 bp upstream of the predicted translation start site. The upstream promoter region contained a sequence (TAAAAT) homologous to the -10 region of the bacterial deltasup 7sup 0-dependent promoters at an appropriate distance (7 bp), but no sequence resembling the consensus sequence of the -35 region was found. These studies demonstrate the ability to use complementation of defined LPS defects in members of the family Enterobacteriaceae to identify LOS synthesis genes in NTHi.

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Devi, S.
09/077572

Key Terms

(FILE 'USPAT' ENTERED AT 10:44:05 ON 01 MAR 1999)
L1 26 SEA HTRB OR HTR B
L2 1 SEA L1(5A)(MUTAT? OR MUTANT# OR MUTAGEN? OR POLYMORPH? OR POLY MORPHI###)
L3 10 SEA L1 AND (COLI OR GRAM(W)(NEGATIVE OR NEG) OR SALMONELL? OR HAEMOPHIL? OR HEMOPHIL?)
L4 10 SEA L2 OR L3

US PAT NO: 5,846,723 [IMAGE AVAILABLE] L4: 1 of 10
TITLE: Methods for detecting the RNA component of telomerase
DATE ISSUED: Dec. 8, 1998
INVENTOR: Nam Woo Kim, San Jose, CA
Fred Wu, San Carlos, CA
James T. Kealey, San Anselmo, CA
Ronald Pruzan, Palo Alto, CA
Scott L. Weinrich, Redwood City, CA
SEARCH-FLD: 435/6, 91.2, 91.3; 536/23.1, 24.3-24.33

ABSTRACT:
Methods of detecting the RNA component of telomerase, diagnosing cancer, and determining its prognosis using polynucleotides that hybridize to the RNA component of mammalian telomerase in a sample.

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L4: 2 of 10
TITLE: Shigella vector for delivering DNA to a mammalian cell
DATE ISSUED: Oct. 20, 1998
INVENTOR: Arthur A. Branstrom, Rockville, MD
Donata R. Sizemore, Gaithersburg, MD
Jerald C. Sadoff, Washington, DC
SEARCH-FLD: 424/234.1, 235.1, 93.2; 435/245, 172.3, 252.1, 252.3, 822, 172.1

ABSTRACT:
We describe a bacterial delivery system for the delivery of DNA and antigens into cells. We constructed an attenuated bacterial vector which enters mammalian cells and ruptures delivering functional plasmid DNA, such as a mammalian expression plasmid, and antigens into the cell cytoplasm. This Shigella vector was designed to deliver DNA to colonic surfaces, thus opening the possibility of oral and other mucosal DNA immunization and gene therapy strategies. The attenuated Shigella is also useful as a vaccine for reducing disease symptoms caused by Shigella.

US PAT NO: 5,801,233 [IMAGE AVAILABLE] L4: 3 of 10
TITLE: Nucleic acid compositions encoding acetyl-coa carboxylase and uses therefor
DATE ISSUED: Sep. 1, 1998
INVENTOR: Robert Haselkorn, Chicago, IL
Piotr Gornicki, Chicago, IL
SEARCH-FLD: 435/172.3, 69.1, 320.1, 252.1, 255.1, 257.1, 325, 410, 419, 6, 975; 536/23.1, 23.2, 23.4, 23.6, 23.7, 24.3, 24.32; 935/9, 22, 66, 60, 67, 73

Searcher : Shears 308-4994

ABSTRACT:
The present invention provides isolated and purified polynucleotides that encode plant and cyanobacterial polypeptides that participate in the carboxylation of acetyl-CoA. Isolated cyanobacterial and plant polypeptides that catalyze acetyl-CoA carboxylation are also provided. Processes for altering acetyl-CoA carboxylation, increasing herbicide resistance of plants and identifying herbicide resistant variants of acetyl-CoA carboxylase are also provided.

US PAT NO: 5,776,679 [IMAGE AVAILABLE] L4: 4 of 10
TITLE: Assays for the DNA component of human telomerase
DATE ISSUED: Jul. 7, 1998
INVENTOR: Bryant Villeponteau, San Carlos, CA
Junli Feng, San Carlos, CA
Walter Funk, Union City, CA
William H. Andrews, Richmond, CA
SEARCH-FLD: 435/6, 91.2, 91.21, 91.51; 436/63, 64; 536/24.31, 23.1, 24.33; 935/8, 3, 78

ABSTRACT:
Nucleic acids comprising the RNA component of a mammalian telomerase are useful as pharmaceutical, therapeutic, and diagnostic reagents.

US PAT NO: 5,599,904 [IMAGE AVAILABLE] L4: 5 of 10
TITLE: Chimeric steroid hormone superfamily receptor proteins
DATE ISSUED: Feb. 4, 1997
INVENTOR: Ronald M. Evans, La Jolla, CA
Estelita S. Ong, San Diego, CA
Prudimar S. Segui, San Diego, CA
Catherine C. Thompson, La Jolla, CA
Kazuhiko Umesono, San Diego, CA
Vincent Giguere, Etobicoke, Canada
SEARCH-FLD: 530/350, 358, 399; 435/69.7, 69.1; 935/36

ABSTRACT:
A novel retinoic acid receptor is disclosed. The novel receptor is encoded for by cDNA carried on plasmid phRAR1, which has been deposited with the American Type Culture Collection for patent purposes. Chimeric receptor proteins are also disclosed. The chimera are constructed by exchanging functional domains between the glucocorticoid, the mineralocorticoid, the estrogen-related, the thyroid and the retinoic acid receptors. In addition, a novel method for identifying functional ligands for receptor proteins is disclosed. The method, which takes advantage of the modular structure of the hormone receptors and the idea that the functional domains may be interchangeable, replaces the DNA-binding domain of a putative novel receptor with the DNA-binding domain of a known receptor such as the glucocorticoid receptor. The resulting chimeric construction, when expressed in cells, produces a hybrid receptor whose activation of a ligand-(e.g., glucocorticoid) inducible promoter is dependent on the presence of the new ligand. The

novel method is illustrated in part by showing that the ligand for the new receptor protein is the retinoid, retinoic acid.

US PAT NO: 5,571,692 [IMAGE AVAILABLE] L4: 6 of 10
TITLE: Retinoic acid receptor .alpha., vectors and cells comprising the same DNA encoding
DATE ISSUED: Nov. 5, 1996
INVENTOR: Ronald M. Evans, La Jolla, CA
Estelita S. Ong, San Diego, CA
Prudimar S. Segui, San Diego, CA
Catherine C. Thompson, La Jolla, CA
Kazuhiko Umesono, San Diego, CA
Vincent Giguere, Etobicoke, Canada
SEARCH-FLD: 536/23.5, 23.4; 435/240.2, 320.1, 252.3, 254.11, 69.1, 69.2

ABSTRACT:
DNA encoding a human retinoic acid receptor alpha protein is disclosed. The sequence of the receptor is encoded by the cDNA insert of plasmid phRAR1, which has been deposited with ATCC. Methods employing chimeric receptors derived from the retinoic acid receptor are illustrated which demonstrate that the ligand for the new receptor is the retinoid, retinoic acid.

US PAT NO: 5,548,063 [IMAGE AVAILABLE] L4: 7 of 10
TITLE: Retinoic acid receptor alpha proteins
DATE ISSUED: Aug. 20, 1996
INVENTOR: Ronald M. Evans, La Jolla, CA
Estelita S. Ong, San Diego, CA
Prudimar S. Segui, San Diego, CA
Catherine C. Thompson, La Jolla, CA
Kazuhiko Umesono, San Diego, CA
Vincent Giguere, Etobicoke, Canada
SEARCH-FLD: 530/350, 324; 435/69.1

ABSTRACT:
A human retinoic acid receptor alpha protein is disclosed. The receptor is encoded by the cDNA insert of plasmid phRAR1, which has been deposited with ATCC. Methods employing chimeric receptors derived from the retinoic acid receptor are illustrated which demonstrate that the ligand for the new receptor is the retinoid, retinoic acid.

US PAT NO: 5,274,077 [IMAGE AVAILABLE] L4: 8 of 10
TITLE: Retinoic acid receptor composition
DATE ISSUED: Dec. 28, 1993
INVENTOR: Ronald M. Evans, La Jolla, CA
Estelita S. Ong, San Diego, CA
Prudimar S. Segui, San Diego, CA
Catherine C. Thompson, La Jolla, CA
Kazuhiko Umesono, San Diego, CA
Vincent Giguere, Etobicoke, Canada
SEARCH-FLD: 530/350, 358; 435/69.7, 252.3

ABSTRACT:
A novel retinoic acid receptor is disclosed. The novel receptor is encoded for by CDNA carried on plasmid phRAR1, which has been deposited with the American Type Culture Collection for patent purposes. Chimeric receptor proteins are also disclosed. The chimera are constructed by exchanging functional domains between the glucocorticoid, the mineralocorticoid, the estrogen-related, the thyroid and the retinoic acid receptors. In addition, a novel method for identifying functional ligands for receptor proteins is disclosed. The method, which takes advantage of the modular structure of the hormone receptors and the idea that the functional domains may be interchangeable, replaces the DNA-binding domain of a putative novel receptor with the DNA-binding domain of a known receptor such as the glucocorticoid receptor. The resulting chimeric construction, when expressed in cells, produces a hybrid receptor whose activation of a ligand--(e.g., glucocorticoid) inducible promoter is dependent on the presence of the new ligand. The novel method is illustrated in part by showing that the ligand for the new receptor protein is the retinoid, retinoic acid.

US PAT NO: 5,171,671 [IMAGE AVAILABLE] L4: 9 of 10
TITLE: Retinoic acid receptor composition
DATE ISSUED: Dec. 15, 1992
INVENTOR: Ronald M. Evans, La Jolla, CA
Estelita S. Ong, San Diego, CA
Prudimar S. Segui, San Diego, CA
Catherine C. Thompson, La Jolla, CA
Kazuhiko Uemsono, San Diego, CA
Vincent Giguere, Etobicoke, Canada
SEARCH-FLD: 536/27; 435/69.1, 69.7, 252.3, 320.1; 530/350

ABSTRACT:
A novel retinoic acid receptor is disclosed. The novel receptor is encoded for by cDNA carried on plasmid phRAR1, which has been deposited with the American Type Culture Collection for patent purposes. Chimeric receptor proteins are also disclosed. The chimera are constructed by exchanging functional domains between the glucocorticoid, the mineralocorticoid, the estrogen-related, the thyroid and the retinoic acid receptors. In addition, a novel method for identifying functional ligands for receptor proteins is disclosed. The method, which takes advantage of the modular structure of the hormone receptors and the idea that the functional domains may be interchangeable, replaces the DNA-binding domain of a putative novel receptor with the DNA-binding domain of a known receptor such as the glucocorticoid receptor. The resulting chimeric construction, when expressed in cells, produces a hybrid receptor whose activation of a ligand-(e.g., glucocorticoid) inducible promoter is dependent on the presence of the new ligand. The novel method is illustrated in part by showing that the ligand for the new receptor protein is the retinoid, retinoic acid.

US PAT NO: 4,981,784 [IMAGE AVAILABLE] L4: 10 of 10

TITLE: Retinoic acid receptor method
DATE ISSUED: Jan. 1, 1991
INVENTOR: Ronald M. Evans, La Jolla, CA
Estelita Ong, San Diego, CA
Prudimar S. Segui, San Diego, CA
Catherine C. Thompson, La Jolla, CA
Kazuhiko Umesono, San Diego, CA
Vincent Giguere, Etobicoke, Canada
SEARCH-FLD: 435/6, 29, 41, 172.1, 172.3, 320, 69.1, 69.4, 69.7, 70.1; 935/6, 11, 9, 13, 23, 27, 70, 76, 111

ABSTRACT:
A novel retinoic acid receptor is disclosed. The novel receptor is encoded for by cDNA carried on plasmid phRAR1, which has been deposited with the American Type Culture Collection for patent purposes. Chimeric receptor proteins are also disclosed. The chimera are constructed by exchanging functional domains between the glucocorticoid, the mineralocorticoid, the estrogen-related, the thyroid and the retinoic acid receptors. In addition, a novel method for identifying functional ligands for receptor proteins is disclosed. The method, which takes advantage of the modular structure of the hormone receptors and the idea that the functional domains may be interchangeable, replaces the DNA-binding domain of a putative novel receptor with the DNA-binding domain of a known receptor such as the glucocorticoid receptor. The resulting chimeric construction, when expressed in cells, produces a hybrid receptor whose activation of a ligand-(e.g., glucocorticoid) inducible promoter is dependent on the presence of the new ligand. The novel method is illustrated in part by showing that the ligand for the new receptor protein is the retinoid, retinoic acid.

=> d his l5-

(FILE 'USPAT' ENTERED AT 10:44:05 ON 01 MAR 1999)
L5 3 S APICELLA, M?/IN
L6 1 S SUNSHINE, M?/IN
L7 250 S LEE, N?/IN
L8 0 S ARUMUGHAM, R?/AU
L9 27 S GIBSON, B?/IN
L10 0 S L5 AND L6 AND L7 AND L9
L11 0 S L5 AND (L6 OR L7 OR L9)
L12 0 S L6 AND (L7 OR L9)
L13 0 S L7 AND L9
L14 0 S (L5 OR L6 OR L7 OR L9) AND L1

-Author(s)

=> fil hom

FILE 'HOME' ENTERED AT 10:49:35 ON 01 MAR 1999

Searcher : Shears 308-4994

Devi, S.
09/077572

-key terms

FILE 'CAPLUS' ENTERED AT 10:23:40 ON 01 MAR 1999

L1 17 SEA ABB=ON PLU=ON (HTRB OR HTR B)(S)(MUTAT? OR MUTAGEN? OR MUTANT OR POLYMORPH? OR POLY(W)MORPHI###)

L2 17 SEA ABB=ON PLU=ON L1 AND (GRAM(W)(NEGATIVE OR NEG) OR SALMONELL? OR COLI OR HAEMOPHIL? OR HEMOPHIL?)

L2 ANSWER 1 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1999:52080 CAPLUS
TI Lipopolysaccharide from an Escherichia **coli htrB** msbB **mutant** induces high levels of MIP-1.alpha. and MIP-1.beta. secretion without inducing TNF-.alpha. and IL-1.beta.
SO J. Hum. Virol. (1998), 1(4), 251-256
CODEN: JHVIFC; ISSN: 1090-9508
AU Hone, David M.; Powell, Jan; Crowley, Richard W.; Maneval, David; Lewis, George K.
PY 1998
AB To identify a lipopolysaccharide (LPS) that retains the capacity to induce .beta.-chemokine secretion without the concomitant activation of pyrogenic cytokines. LPS was extd. from strain MLK986 (mLPS), an htrB1::Tn10, msbB::ocam mutant of Escherichia **coli** that is defective for lipid A synthesis, and from wild-type parent E **coli** strains, W3110 (wtLPS). The capacity of these LPS prepns. to induce tumor necrosis factor-.alpha. (TNF-.alpha.), interleukin-1.beta. (IL-1.beta.), and macrophage inflammatory proteins 1.alpha. (MIP-1.alpha.) and MIP-1.beta. was assessed using a human peripheral blood mononuclear cell (PBMC) activation assay. Stimulation of PBMCs with mLPS did not induce measurable levels of pyrogenic cytokines TNF-.alpha. and IL-1.beta., whereas wtLPS induced high levels of these cytokines. Furthermore, mLPS antagonized the induction of TNF-.alpha. secretion by wtLPS. Nonetheless, mLPS retained a discrete agonist activity that induced MIP-1.alpha. and MIP-1.beta. secretion by PBMCs. This latter agonist activity appears to be unique to mLPS, since two previously documented LPS antagonists, Rhodobacter sphaeroides diphosphoryl lipid A and synthetic lipid IVA, did not induce MIP-1.alpha. and MIP-1.beta. secretion. Furthermore, synthetic lipid IVA was an antagonist of MIP-1.alpha. and MIP-1.beta. induction by mLPS. These results show that mLPS exhibits a novel bipartite activity, being an effective antagonist of TNF-.alpha. induction by wtLPS, while paradoxically being an agonist of MIP-1.alpha. and MIP-1.beta. secretion.

L2 ANSWER 2 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1998:543176 CAPLUS
DN 129:159061
TI **Salmonella** lacking lipid A as a result of **mutation** in the msbB or **htrB** genes
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2

IN Maskell, Duncan John; Dougan, Gordon

APPLICATION NO.	DATE
WO 98-GB291	19980130
AU 98-58734	19980130

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9833923	A1	19980806	WO 98-GB291	19980130
AU 9858734	A1	19980825	AU 98-58734	19980130

PI WO 9833923 A1 19980806 WO 98-GB291 19980130
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9858734 A1 19980825 AU 98-58734 19980130

PY 1998
1998

AB Two genes, msbB and **htrB**, involved in lipid A biosynthesis in **Salmonella** are cloned from S. typhimurium and **mutations** in them are used to create lipid A-deficient strains of **Salmonella**. The lipid A-deficient strains are less toxigenic than wild-type strains and may be used in vaccines. These microorganisms may also be useful as expression hosts. The lipopolysaccharide from these mutants may also be of use as an endotoxin antagonist. Inoculation of mice with an msbB mutant of S. typhimurium led to .apprx.5% fatalities within 7 days compared with 100% fatality in a group inoculated with the wild-type microorganism. The mutants were less effective at induction of synthesis of tumor necrosis factor .alpha. and interleukin 1.beta. in vitro than were control strains. Double mutant strains (aro/msbB) gave 40% protection when used in an oral vaccine compared to 100% protection for an aroA single mutant.

L2 ANSWER 3 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1998:471252 CAPLUS
DN 129:200426
TI Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by Pseudomonas fluorescens WCS365
SO Mol. Plant-Microbe Interact. (1998), 11(8), 763-771
CODEN: MPMIEL; ISSN: 0894-0282
AU Dekkers, Linda C.; Van Der Bij, Arjan J.; Mulders, Ine H. M.; Phoelich, Claartje C.; Wentwoord, Rino A. R.; Glandorf, Deborah C. M.; Wijffelman, Carel A.; Lugtenberg, Ben J. J.
PY 1998

AB Colonization-defective, transposon-induced mutants of the efficient root colonizer Pseudomonas fluorescens WCS365 were identified with a gnotobiotic system. Most mutants were impaired in known colonization traits, i.e., prototrophy for amino acids, motility, and synthesis of the O-antigen of LPS (lipopolysaccharide). Mutants lacking the O-antigen of LPS were impaired in both colonization and competitive growth, whereas one mutant (PCL1205) with a shorter O-antigen chain was defective only in colonization ability, suggesting a role for the intact O-antigen of LPS in colonization. Eight competitive colonization mutants that were not defective in the above-mentioned traits colonized the tomato root tip well when inoculated alone, but were defective in competitive root colonization of tomato, radish, and wheat, indicating they contained mutations affecting host range. One of these eight mutants (PCL1201) was further characterized and contains a mutation in a gene that shows homol. to the Escherichia **coli** nuo4 gene, which encodes a subunit of one of two known NADH:ubiquinone oxidoreductases. Competition expts. in an oxygen-poor medium between mutant PCL1201 and its parental strain showed a decreased growth rate of mutant PCL1201. The requirement of the nuo4 gene homolog for optimal growth under conditions of oxygen limitation suggests that the root-tip environment is microaerobic. A **mutant** characterized by a slow growth rate (PCL1216) was analyzed further and contained a **mutation** in a gene with similarity to the E. **coli HtrB** protein, a lauroyl transferase that functions in lipid A biosynthesis.

L2 ANSWER 4 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1998:330380 CAPLUS
DN 129:65382
TI Function of Escherichia **coli** MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis
SO J. Biol. Chem. (1998), 273(20), 12466-12475
CODEN: JBCHA3; ISSN: 0021-9258
AU Zhou, Zhimin; White, Kimberly A.; Polissi, Alessandra; Georgopoulos, Costa; Raetz, Christian R. H.
PY 1998
AB The Escherichia **coli** msbA gene, first identified as a multicopy suppressor of **htrB mutations**, has been proposed to transport nascent core-lipid A mols. across the inner membrane. MsbA is an essential E. **coli** gene with high sequence similarity to mammalian Mdr proteins and certain types of bacterial ABC transporters. HtrB is required for growth above 32.degree.C and encodes the lauroyltransferase that acts after Kdo addn. during lipid A biosynthesis. By using a quant. new 32Pi labeling technique, the authors demonstrate that hexa-acylated species of lipid A predominate in the outer membranes of wild type E. **coli** labeled for several generations at 42.degree.C. In contrast, in **htrB mutants** shifted to

42.degree.C for 3 h, tetraacylated lipid A species and glycerophospholipids accumulate in the inner membrane. Extra copies of the cloned msbA gene restore the ability of **htrB mutants** to grow at 42.degree.C, but they do not increase the extent of lipid A acylation. However, a significant fraction of the tetra-acylated lipid A species that accumulate in **htrB mutants** are transported to the outer membrane in the presence of extra copies of msbA. E. **coli** strains in which msbA synthesis is selectively shut off at 42.degree.C accumulate hexa-acylated lipid A and glycerophospholipids in their inner membranes. These results suggest that MsbA plays a role in lipid A and possibly glycero-phospholipid transport. The tetra-acylated lipid A precursors that accumulate in **htrB mutants** may not be transported as efficiently by MsbA as are penta- or hexa-acylated lipid A species.

L2 ANSWER 5 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1997:731002 CAPLUS
DN 128:20429
TI Study of the role of the htrB gene in **Salmonella** typhimurium virulence
SO Infect. Immun. (1997), 65(11), 4778-4783
CODEN: INFIBR; ISSN: 0019-9567
AU Jones, Bradely D.; Nichols, Wade A.; Gibson, Bradford W.; Sunshine, Melvin G.; Apicella, Michael A.
PY 1997
AB We have undertaken a study to investigate the contribution of the htrB gene to the virulence of pathogenic **Salmonella** typhimurium. An **htrB**::mini-Tn10 **mutation** from Escherichia **coli** was transferred by transduction to the mouse-virulent strain S. typhimurium SL1344 to create an **htrB mutant**. The S. typhimurium **htrB mutant** was inoculated into mice and found to be severely limited in its ability to colonize organs of the lymphatic system and to cause systemic disease in mice. A variety of expts. were performed to det. the possible reasons for this loss of virulence. Serum killing assays revealed that the S. typhimurium **htrB mutant** was as resistant to killing by complement as the wild-type strain. However, macrophage survival assays revealed that the S. typhimurium **htrB mutant** was more sensitive to the intracellular environment of murine macrophages than the wild-type strain. In addn., the bioactivity of the lipopolysaccharide (LPS) of the **htrB mutant** was reduced compared to that of the LPS from the parent strain as measured by both a Limulus amoebocyte lysate endotoxin quantitation assay and a tumor necrosis factor alpha bioassay. These results indicated that the htrB gene plays a role in the virulence of S. typhimurium.

L2 ANSWER 6 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1997:730328 CAPLUS
DN 128:21296
TI Evaluation of the virulence of nontypeable **Hemophilus** influenzae lipooligosaccharide **htrB** and rfaD **mutants** in the chinchilla model of otitis media
SO Infect. Immun. (1997), 65(11), 4431-4435
CODEN: INFIBR; ISSN: 0019-9567
AU DeMaria, T. F.; Apicella, M. A.; Nichols, W. A.; Leake, E. R.
PY 1997
AB Considerable evidence has implicated nontypeable **Hemophilus** influenzae (NTHi) lipooligosaccharide (LOS) in the pathogenesis of otitis media (OM); however, its exact role has not been conclusively established. Recently, two NTHi LOS-deficient mutants have been created and described. Strain 2019-DK1, an rfaD gene mutant, expresses a truncated LOS consisting of only three deoxy-D-manno-octulosonic acid residues, a single heptose, and lipid A. Strain 2019-B29, an isogenic **htrB mutant**, possesses an altered oligosaccharide core and an altered lipid A. Each strain's ability to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation was evaluated in the chinchilla model. Nasopharyngeal colonization data indicate that the parent strain and both mutants are able to colonize the nasopharynx and exhibit comparable clearance kinetics. Compared with the parent and each other, however, the mutants demonstrated marked differences in virulence regarding their relative abilities to induce OM and persist in the middle ear post-transbullar inoculation. Strain B29 required a 3-log-greater dose to induce OM than the parent strain and did not exhibit evidence of sustained multiplication but persisted for the same duration as the parent. Conversely, strain-DK1, even when inoculated at a dose 4 logs greater than the parent dose, was eliminated from the middle ear 72 h after challenge. A comparison of the relative pathogenicities of these isolates provides the opportunity to address fundamental questions regarding the contribution of LOS to pathogenesis issues at the mol. level. Specifically, the impact of these LOS gene disruptions on OM pathogenesis can be defined and may thus provide potential new targets for future protection and intervention strategies.

L2 ANSWER 7 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1997:605036 CAPLUS
DN 127:275201
TI htrB of **Haemophilus** influenzae: determination of biochemical activity and effects on virulence and lipooligosaccharide toxicity
SO J. Endotoxin Res. (1997), 4(3), 163-172
CODEN: JENREB; ISSN: 0968-0519
AU Nichols, W. A.; Raetz, C. R. H.; Clementz, T.; Smith, A. L.; Hanson,

J. A.; Ketterer, M. R.; Sunshine, M.; Apicella, M. A.
PY 1997
AB The **htrB mutant** of **Haemophilus** influenzae (strain B29) has been shown to lack secondary (nonhydroxylated) acyl groups in its lipid A. The authors have detd. through in vitro biochem. assays that the HtrB protein acts as a specific acyltransferase in the late stages of lipid A biosynthesis and that the preferred acyl group donor is myristoyl-acyl carrier protein. Under the conditions employed, the Escherichia **coli** precursor, Kdo2-lipid IVA, functions as a myristate acceptor. Introduction of the **Haemophilus htrB** gene into an E. **coli mutant** lacking **htrB** complements the biochem. and physiol. defects assocd. with the E. **coli htrB mutation**. Tumor necrosis factor .alpha. (TNF.alpha.) assays using murine and human macrophage cells indicated that nontypeable H. influenzae (NtHi) strain 2019 and H. influenzae type b strain A2 elicit levels of expression of TNF.alpha. that are 30-40 times greater than levels induced by the isogenic **htrB mutants** (B29 and A2B29). Studies using cell-free LOS indicated that the LOS from wild type strain 2019 elicits levels of TNF.alpha. expression that are 6-8-fold higher than those of B29. In situ hybridization studies of a primary human bronchial epithelial cell line demonstrated a greater increase of TNF.alpha. message produced in the presence of 2019 LOS than in the presence of B29 LOS. TNF.alpha. levels of the cell supernatant of cells stimulated with 2019 LOS were found to be 7-8-fold higher than levels in B29 stimulated supernatants. Using the Limulus amoebocyte lysate for assessment of endotoxic activity, we found that wild type LOS was 8-fold higher in endotoxic activity compared with the mutant LOS. In virulence assays using i.p. inoculation of infant rats, the htrB isogenic strain caused bacteremia at 50% the frequency of the wild type strain. In intranasal inoculation studies, the **htrB mutant** strain was unable to cause bacteremia whereas the wild type b parent produced bacteremia in 40-60% of the animals. These findings suggest that the **htrB** gene of H. influenzae is important for virulence and that host TNF.alpha. expression is attenuated in response to **htrB mutant** strains.

L2 ANSWER 8 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1997:591347 CAPLUS
DN 127:288753
TI **Mutation** of the **htrB** gene in a virulent **Salmonella** typhimurium strain by intergeneric transduction: strain construction and phenotypic characterization
SO J. Bacteriol. (1997), 179(17), 5521-5533
CODEN: JOBAAY; ISSN: 0021-9193
AU Sunshine, Melvin G.; Gibson, Bradford W.; Engstrom, Jeffrey J.; Nichols, Wade A.; Jones, Bradley D.; Apicella, Michael A.

PY 1997
AB The htrB gene product of **Haemophilus** influenzae contributes to the toxicity of the lipooligosaccharide. The htrB gene encodes a 2-keto-3-deoxyoctulosonic acid-dependent acyltransferase which is responsible for myristic acid substitutions at the hydroxy moiety of lipid A .beta.-hydroxymyristic acid. Mass spectroscopic anal. has demonstrated that lipid A from an H. influenzae **htrB mutant** is predominantly tetraacyl and similar in structure to lipid IVA, which has been shown to be nontoxic in animal models. We sought to construct a **Salmonella** typhimurium **htrB mutant** in order to investigate the contribution of **htrB** to virulence in a well-defined murine typhoid model of animal pathogenesis. To this end, an r- m+ galE mutS recD strain of S. typhimurium was constructed (MGS-7) and used in inter- and intrastrain transduction expts. with both coliphage P1 and **Salmonella** phage P22. The Escherichia **coli** htrB gene contg. a mini-Tn10 insertion was transduced from E. **coli** MLK217 into S. typhimurium MGS-7 via phage P1 and subsequently via phage P22 into the virulent **Salmonella** strain SL1344. All S. typhimurium transductants showed phenotypes similar to those described for the E. **coli htrB mutant**. Mass spectrometric anal. of the crude lipid A fraction from the lipopolysaccharide of the S. typhimurium **htrB mutant** strain showed that for the dominant hexaacyl form, a lauric acid moiety was lost at one position on the lipid A and a palmitic acid moiety was added at another position; for the less abundant heptaacyl species, the lauric acid was replaced with palmitoleic acid.

L2 ANSWER 9 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1997:496805 CAPLUS
DN 127:107983
TI Non-toxic mutants of pathogenic **gram-negative** bacteria
SO PCT Int. Appl., 78 pp.
CODEN: PIXXD2
IN Apicella, Michael A.; Sunshine, Melvin G.; Lee, Na-gyong; Arumugham, Rasappa; Gibson, Bradford W.

APPLICATION NO.	DATE
WO 96-US18984	19961127
CA 96-2238640	19961127
AU 97-11246	19961127
EP 96-942080	19961127

AI

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9719688	A1	19970605	WO 96-US18984	19961127

PI W: AU, CA, JP, KR, MX, NZ, US, AM, AZ, BY, KG, KZ, MD, RU, TJ,

TM
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

CA 2238640	AA	19970605	CA 96-2238640	19961127
AU 9711246	A1	19970619	AU 97-11246	19961127
EP 876150	A1	19981111	EP 96-942080	19961127

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PY 1997
1997
1997
1998

AB A method is provided for identifying, isolating, and producing **htrB mutants** of **gram-neg.** bacterial pathogens. The method comprises **mutating** the **htrB** gene of a **gram-neg.** bacterial pathogen so that there is a lack of a functional **htrB** protein, resulting in a **mutant** that lacks .gtoreq.1 secondary acyl chains contained in the wild type **gram-neg.** bacterial pathogen, and displays substantially reduced toxicity as compared to the wild type strain. The present invention also provides methods for using a vaccine formulation contg. the **htrB mutant**, the endotoxin isolated therefrom, or the endotoxin isolated therefrom which is then conjugated to a carrier protein to immunize an individual against infections caused by **gram-neg.** bacterial pathogens by administering a prophylactically effective amt. of the vaccine formulation.

L2 ANSWER 10 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1997:279634 CAPLUS
DN 126:339596
TI Function of the Escherichia **coli** msbB gene, a multicopy suppressor of htrB knockouts, in the acylation of lipid A. Acylation by MsbB follows laurate incorporation by HtrB
SO J. Biol. Chem. (1997), 272(16), 10353-10360
CODEN: JBCHA3; ISSN: 0021-9258
AU Clementz, Tony; Zhou, Zhimin; Raetz, Christian R. H.
PY 1997
AB Overexpression of the Escherichia **coli** msbB gene on high copy plasmids suppresses the temp.-sensitive growth assocd. with **mutations** in the **htrB** gene. HtrB encodes the lauroyl transferase of lipid A biosynthesis that acylates the intermediate (Kdo)2-lipid IVA. Since msbB displays 27.5% identity and 42.2% similarity to htrB, we explored the possibility that msbB encodes a related acyltransferase. In contrast to **htrB**, exts. of strains with insertion **mutations** in msbB are not defective in transferring laurate from lauroyl acyl carrier protein to (Kdo)2-lipid IVA. However, exts. of msbB **mutants** do

not efficiently acylate the product formed by **HtrB**, designated (Kdo)2-(lauroyl)-lipid IVA. Exts. of strains harboring msbB+ bearing plasmids acylate (Kdo)2-(lauroyl)-lipid IVA very rapidly compared with wild type. We solubilized and partially purified msbB from an overproducing strain, lacking HtrB, MsbB transfers myristate or laurate, activated on ACP, to (Kdo)2-(lauroyl)-lipid IVA. Decanoyl, palmitoyl, palmitoleoyl, and (R)-3-hydroxymyristoyl-ACP are poor acyl donors. MsbB acylates (Kdo)2-(lauroyl)-lipid IVA .apprx.100 times faster than (Kdo)2-lipid IVA. The slow, but measurable, rate whereby MsbB acts on (Kdo)2-lipid IVA may explain why overexpression of MsbB suppresses the temp.-sensitive phenotype of **htrB mutations**. Presumably, the acyloxyacyl group generated by excess MsbB substitutes for the one normally formed by HtrB.

L2 ANSWER 11 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1996:411262 CAPLUS
DN 125:134544
TI Mutational analysis and properties of the msbA gene of Escherichia **coli**, coding for an essential ABC family transporter
SO Mol. Microbiol. (1996), 20(6), 1221-1233
CODEN: MOMIEE; ISSN: 0950-382X
AU Polissi, Alessandra; Georgopoulos, Costa
PY 1996
AB The htrB gene was discovered because its insertional inactivation interfered with Escherichia **coli** growth and viability at temps. above 32.5.degree., as a result of accumulation of phospholipids. The msbA gene was originally discovered because when cloned on a low-cop-no. plasmid vector it was able to suppress the temp.-sensitive growth phenotype of an **htrB** null **mutant** as well as the accumulation of phospholipids. The msbA gene product belongs to the superfamily of ABC transporters, a universally conserved family of proteins characterized by a highly conserved ATP-binding domain. The msbA gene is essential for bacterial viability at all temps. To understand the physiol. role of the MsbA protein, we mutated the ATP-binding domain using random PCr mutagenesis. Six independent mutants were isolated and characterized. Four of these mutations resulted in single-amino-acid substitutions in non-conserved residues and were able to support cell growth at 30.degree. but not at 43.degree.. The remaining two mutations behaved as recessive lethals, and resulted in single-amino-acid substitutions in Walker motif B, one of the two highly conserved regions of the ATP-binding domain. Despite the fact that neither of these two mutant proteins can support E. **coli** growth, they both retained the ability to bind ATP in vitro. In addn., we present evidence to show that N-acetyl [3H]-glucosamine, a precursor of lipopolysaccharides, accumulates at the non-permissive temp. in the inner membrane of either htrB null or msbA conditional lethal strains. Translocation

of the precursor to the outer membrane is restored by transformation with a plasmid contg. the wild-type msbA gene. A possible role for MsbA as a translocator of lipopolysaccharides or its precursors is discussed.

L2 ANSWER 12 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1995:945651 CAPLUS
DN 124:25327
TI **Mutation** of the **htrB** locus of **Haemophilus** influenzae nontypable strain 2019 is associated with modifications of lipid A and phosphorylation of the lipo-oligosaccharide
SO J. Biol. Chem. (1995), 270(45), 27151-9
CODEN: JBCHA3; ISSN: 0021-9258
AU Lee, Na-Gyong; Sunshine, Melvin G.; Engstrom, Jeffery J.; Gibson, Bradford W.; Apicella, Michael A.
PY 1995
AB The HtrB protein was first identified in Escherichia **coli** as a protein required for cell viability at high temp., but its expression was not regulated by temp. An **htrB** homolog was isolated from nontypable **Haemophilus** influenzae strain (NTHi) 2019, which was able to functionally complement the E. **coli htrB mutation**. The promoter for the NTHi 2019 htrB gene overlaps the promoter for the rfaE gene, and the 2 genes are divergently transcribed. The deduced amino acid sequence of NTHi 2019 HtrB had 56% homol. to E. **coli** HtrB. In vitro transcription-translation anal. confirmed prodn. of a protein with an apparent mol. mass of 32-33 kDa. Primer extension anal. revealed that htrB was transcribed from a .sigma.70-dependent consensus promoter and its expression was not affected by temp. The expression of **htrB** and rfaE was 2.5-4-fold higher in the NTHi **htrB mutant** B29 than in the parental strain. In order to study the function of the **HtrB** protein in **Haemophilus**, 2 isogenic **htrB mutants** were generated by shuttle **mutagenesis** using a mini-Tn3. The **htrB mutants** initially showed temp. sensitivity, but they lost the sensitivity after a few passages at 30.degree. and were able to grow at 37.degree.. They also showed hypersensitivity to deoxycholate and kanamycin, which persisted on passage. SDS-PAGE anal. revealed that the lipo-oligosaccharide (LOS) isolated from these **mutants** migrated faster than the wild type LOS and its color changed from black to brown as has been described for E. **coli htrB mutants**. Immunoblotting anal. also showed that the LOS from the **htrB mutants** lost reactivity to a monoclonal antibody, 6E4, which binds to the wild type NTHi 2019 LOS. Electrospray ionization-mass spectrometry anal. of the O-deacylated LOS oligosaccharide indicated a modification of the core structure characterized in part by a net loss in

phosphoethanolamine. Mass spectrometric anal. of the lipid A of the **htrB mutant** indicated a loss of one or both myristic acid substitutions. These data suggest that HtrB is a multifunctional protein and may play a controlling role in regulating cell responses to various environmental changes.

L2 ANSWER 13 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1993:487844 CAPLUS
DN 119:87844
TI The essential Escherichia **coli** msbA gene, a multicopy suppressor of null **mutations** in the **htrB** gene, is related to the universally conserved family of ATP-dependent translocators
SO Mol. Microbiol. (1993), 7(1), 69-79
CODEN: MOMIEE; ISSN: 0950-382X
AU Karow, Margaret; Georgopoulos, Costa
PY 1993
AB The msbA gene, isolated as a multicopy suppressor of the HtrB temp.-sensitive phenotype, was characterized. The msbA gene maps to 20.5 min on the Escherichia **coli** genetic map and encodes a protein with an estd. mol. mass of 64,460 Da, with the properties of an integral membrane protein. The amino acid sequence of MsbA is very similar to those of the family of ATP-dependent translocators, which includes the hemolysin B protein of E. **coli** and the mammalian multidrug resistance (MDR) proteins. Mutational anal. of msbA indicates that it may form an operon with a down-stream gene, orfE, and that both of these genes are essential for bacterial viability under all growth conditions tested.

L2 ANSWER 14 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1993:162023 CAPLUS
DN 118:162023
TI Isolation and characterization of the Escherichia **coli** msbB gene, a multicopy suppressor of null **mutations** in the high-temperature requirement gene **htrB**
SO J. Bacteriol. (1992), 174(3), 702-10
CODEN: JOBAAY; ISSN: 0021-9193
AU Karow, Margaret; Georgopoulos, Costa
PY 1992
AB Previous work established that the htrB gene of E. **coli** is required for growth in rich media at temps. about 32.5.degree. but not at lower temps. In an effort to det. the functional role of the htrB gene product, the authors isolated a multicopy suppressor of htrB, called msbB. The msbB gene has been mapped to 40.5 min on the E. **coli** genetic map, in a 12- to 15-kb gap of the genomic library made by Y. Kohara et al. (1987). Mapping data show that the order of genes in the region is eda-edd-zwf-pykA-msbB. The msbB gene codes for a protein of 37,410 Da whose amino acid sequence is similar to that of HtrB and, like HtrB, the protein is very basic in

nature. The similarity of the HtrB and MsbB proteins may indicate that they play functionally similar roles. **Mutational** anal. of msbB shows that the gene is not essential for E. **coli** growth; however, the **htrB** msbB double **mutant** exhibits a unique morphol. phenotype at 30.degree. not seen with either of the single **mutants**. Anal. of both msbB and **htrB mutants** shows that these bacteria are resistant to 4-fold more deoxycholate than are wild-type bacteria but not to other hydrophobic substances. The addn. of quaternary ammonium compds. rescues the temp.-sensitive phenotype of htrB bacteria, and this rescue is abolished by the simultaneous addn. of Mg2+ or Ca2+. These results suggest that MsbB and HtrB play an important role in outer membrane structure and/or function.

L2 ANSWER 15 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1993:19016 CAPLUS
DN 118:19016
TI The lethal phenotype caused by null **mutations** in the Escherichia **coli htrB** gene is suppressed by **mutations** in the accBC operon, encoding two subunits of acetyl coenzyme A carboxylase
SO J. Bacteriol. (1992), 174(22), 7407-18
CODEN: JOBAAY; ISSN: 0021-9193
AU Karow, Margaret; Fayet, Olivier; Georgopoulos, Costa
PY 1992
AB Insertion **mutations** in the E. **coli htrB** gene result in the unique phenotype of not affecting growth at temps. <32.5.degree. but leading to a loss of viability at temps. above this in rich media. When htrB bacteria growing in rich media were shifted to the nonpermissive temp. of 42.degree., they continued to grow at a rate similar to that of 30.degree. but they produced phospholipids at the rate required for growth at 42.degree.. This led to the accumulation of more than twice as much phospholipid per mg of protein compared with that in wild-type bacteria. Consistent with **HtrB** playing a role in phospholipid biosynthesis, one complementation group of spontaneously arising **mutations** that suppressed **htrB**-induced lethality were mapped to the accBC operon. This operon codes for the biotin carboxyl carrier protein and biotin carboxylase subunits of the acetyl CoA carboxylase enzyme complex, which catalyzes the 1st step in fatty acid biosynthesis. Four suppressor mutations mapped to this operon. Two alleles were identified as mutations in the accC gene, the 3rd allele was identified as a mutation in the accB gene, and the 4th as an insertion of an IS1 transposable element in the promoter region of the operon, resulting in reduced transcription. The suppressor **mutations** caused a decrease in the rate of phospholipid biosynthesis, restoring the balance between the biosynthesis of phospholipids and growth rate, thus enabling **htrB** bacteria

to grow at high temps.

L2 ANSWER 16 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1992:525535 CAPLUS
DN 117:125535
TI Sequencing, **mutational** analysis, and transcriptional regulation of the Escherichia **coli htrB** gene
SO Mol. Microbiol. (1991), 5(9), 2285-92
CODEN: MOMIEE; ISSN: 0950-382X
AU Karow, M.; Georgopoulos, C.
PY 1991
AB The E. **coli** htrB gene was originally discovered because its insertional inactivation led to an exquisitely temp.-sensitive phenotype in rich media, i.e. the ability to form colonies at temps. below 32.degree., but not above 33.degree.. The htrB gene has been sequenced. It can potentially code for 2 proteins, with Mr values of 35407 Da and 8669 Da, that are encoded by overlapping, divergent open reading frames. The data are consistent with the 35407 Da protein being HtrB. Norther blot anal. clearly shows that the monocistronic htrB message is not under heat-shock regulation. The flanking DNA was also sequenced and a new gene, designated orf39.9, located immediately adjacent to htrB, but divergently transcribed was discovered.

L2 ANSWER 17 OF 17 CAPLUS COPYRIGHT 1999 ACS
AN 1992:102492 CAPLUS
DN 116:102492
TI Complex phenotypes of null mutations in the htr genes, whose products are essential for Escherichia **coli** growth at elevated temperatures
SO Res. Microbiol. (1991), 142(2-3), 289-94
CODEN: RMCREW; ISSN: 0923-2508
AU Karow, M.; Raina, S.; Georgopoulos, C.; Fayet, O.
PY 1991
AB Transposon insertion, followed by screening, has allowed the identification of a set of genes, called htr, whose products are required for E. **coli** growth at elevated temps. The htrB gene maps at 23.5 min on the E. **coli** genetic map. It codes for a very basic, hydrophobic, 35,000-Mr polypeptide, possessing a putative membrane-spanning domain. At nonpermissive temp., **htrB mutant** bacteria stop dividing, followed by the formation of bulges and eventual lysis. The htrC gene maps at 90 min, is under .sigma.32 regulation, and codes for a 21,130-Mr polypeptide. At 43.degree. htrC mutant bacteria gradually lyse, whereas at intermediate temps. they filament extensively. Finally, the htrM gene maps at 81 min, is under .sigma.32 regulation, and codes for a 35,000-Mr polypeptide. The HtrM null phenotype included inability to grow at >42.degree., extreme mucoidness, and sensitivity to bile salts, even at the permissive

temps. The htrM gene is identical to the rfaD gene, whose product is required for the biosynthesis of the lipopolysaccharide precursor ADP-L-glycero-D-mannoheptose.

=> d his l3- ful; d 1-21 bib abs

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, BIOTECHDS, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, DRUGU, DRUGNL, DRUGB' ENTERED AT 10:26:29 ON 01 MAR 1999)

L3 98 SEA ABB=ON PLU=ON L1
L4 98 SEA ABB=ON PLU=ON L3 AND (GRAM(W)(NEGATIVE OR NEG) OR SALMONELL? OR COLI OR HAEMOPHIL? OR HEMOPHIL?)
L5 21 DUP REM L4 (77 DUPLICATES REMOVED)

L5 ANSWER 1 OF 21 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 98-10001 BIOTECHDS
TI New non-functional **mutant** nucleic acid from **Salmonella** msbB or **htrB** genes; lipid-A preparation by **Salmonella** typhimurium msbB, **htrB mutant** gene transfer and expression in host cell, used for e.g. septic shock therapy, gene therapy, or recombinant vaccine
AU Maskell D J; Dougan G
PA Univ.London
LO London, UK.
PI WO 9833923 6 Aug 1998
AI WO 98-GB291 30 Jan 1998
PRAI GB 97-1887 30 Jan 1997; GB 97-1886 30 Jan 1997
DT Patent
LA English
OS WPI: 98-437476 [37]
AN 98-10001 BIOTECHDS
AB A new **Salmonella** bp. msbB or **htrB** gene sequence has a **mutation** that results in the loss of the encoded protein or its activity, and results in a lipid-A molecule of reduced toxicity. Also new are protein encoded by the DNA, a recombinant DNA construct containing the DNA, and a microorganism containing the **mutated** msbB and/or **htrB** gene, where one or both of the genes are inactivated. The microorganisms may be used as live vaccines to protect against infection by the corresponding wild-type, in humans, mammals or birds. The microorganisms may also be used for production of nucleic acid or proteins for therapy or gene therapy, e.g. lipopolysaccharides may be used as endotoxin antagonists for treatment of septic shock. The new DNA is preferably a **mutant** msbB gene derived from **Salmonella** typhimurium C5 (NCIMB 40856), but may also be from one of 18 specified **Salmonella** spp. or from one of 19 specified genera. The **mutant** is produced by inserting a kanamycin-resistance cassette into the gene, conjugating as a

suicide vector into a recipient that is to be **mutated**, and transducing into another recipient. (38pp)

L5 ANSWER 2 OF 21 MEDLINE DUPLICATE 2
AN 1998241619 MEDLINE
DN 98241619
TI Function of Escherichia **coli** MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis.
AU Zhou Z; White K A; Polissi A; Georgopoulos C; Raetz C R
CS Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA.
NC GM-51310 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 May 15) 273 (20) 12466-75. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199808
EW 19980803
AB The Escherichia **coli** msbA gene, first identified as a multicopy suppressor of **htrB mutations**, has been proposed to transport nascent core-lipid A molecules across the inner membrane (Polissi, A., and Georgopoulos, C. (1996) Mol. Microbiol. 20, 1221-1233). msbA is an essential E. **coli** gene with high sequence similarity to mammalian Mdr proteins and certain types of bacterial ABC transporters. **htrB** is required for growth above 32 degreesC and encodes the lauroyltransferase that acts after Kdo addition during lipid A biosynthesis (Clementz, T., Bednarski, J., and Raetz, C. R. H. (1996) J. Biol. Chem. 271, 12095-12102). By using a quantitative new 32Pi labeling technique, we demonstrate that hexa-acylated species of lipid A predominate in the outer membranes of wild type E. **coli** labeled for several generations at 42 degreesC. In contrast, in **htrB mutants** shifted to 42 degreesC for 3 h, tetra-acylated lipid A species and glycerophospholipids accumulate in the inner membrane. Extra copies of the cloned msbA gene restore the ability of **htrB mutants** to grow at 42 degreesC, but they do not increase the extent of lipid A acylation. However, a significant fraction of the tetra-acylated lipid A species that accumulate in **htrB mutants** are transported to the outer membrane in the presence of extra copies of msbA. E. **coli** strains in which msbA synthesis is selectively shut off at 42 degreesC accumulate hexa-acylated lipid A and glycerophospholipids in their inner membranes. Our results support the view that MsbA plays a role in lipid A and possibly glycerophospholipid transport. The tetra-acylated lipid A precursors that accumulate in **htrB mutants** may not be transported as efficiently by MsbA as are penta- or hexa-acylated

lipid A species.

L5 ANSWER 3 OF 21 MEDLINE DUPLICATE 3
AN 1998340543 MEDLINE
DN 98340543
TI Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by Pseudomonas fluorescens WCS365.
AU Dekkers L C; van der Bij A J; Mulders I H; Phoelich C C; Wentwoord R A; Glandorf D C; Wijffelman C A; Lugtenberg B J
CS Leiden University, Institute of Molecular Plant Sciences, Clusius Laboratory, The Netherlands.. Dekkers@rulbim.leidenuniv.nl
SO MOLECULAR PLANT-MICROBE INTERACTIONS, (1998 Aug) 11 (8) 763-71. Journal code: A9P. ISSN: 0894-0282.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-Y14568; GENBANK-Y14569
EM 199810
EW 19981004
AB Colonization-defective, transposon-induced **mutants** of the efficient root colonizer Pseudomonas fluorescens WCS365 were identified with a gnotobiotic system. Most **mutants** were impaired in known colonization traits, i.e., prototrophy for amino acids, motility, and synthesis of the O-antigen of LPS (lipopolysaccharide). **Mutants** lacking the O-antigen of LPS were impaired in both colonization and competitive growth whereas one **mutant** (PCL1205) with a shorter O-antigen chain was defective only in colonization ability, suggesting a role for the intact O-antigen of LPS in colonization. Eight competitive colonization **mutants** that were not defective in the above-mentioned traits colonized the tomato root tip well when inoculated alone, but were defective in competitive root colonization of tomato, radish, and wheat, indicating they contained **mutations** affecting host range. One of these eight **mutants** (PCL1201) was further characterized and contains a **mutation** in a gene that shows homology to the Escherichia **coli** nuo4 gene, which encodes a subunit of one of two known NADH:ubiquinone oxidoreductases. Competition experiments in an oxygen-poor medium between **mutant** PCL1201 and its parental strain showed a decreased growth rate of **mutant** PCL1201. The requirement of the nuo4 gene homolog for optimal growth under conditions of oxygen limitation suggests that the root-tip environment is micro-aerobic. A **mutant** characterized by a slow growth rate (PCL1216) was analyzed further and contained a **mutation** in a gene with similarity to the E. **coli** **HtrB** protein, a lauroyl transferase that functions in lipid

A biosynthesis.

L5 ANSWER 4 OF 21 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 97-08966 BIOTECHDS
TI New **Gram-negative** bacterial pathogen vaccines; **Gram-negative** bacterium **htrB** endotoxin gene **mutagenesis** for reduced toxicity and use as a vaccine
AU Apicella M A; Sunshine M G; Lee N G; Arumugham R; Gibson B W
PA Univ.Iowa-Res.Found.; Univ.California; American-Cyanamid
LO Iowa City, IA, USA; Oakland, CA, USA; Madison, NJ, USA.
PI WO 9719688 5 Jun 1997
AI WO 96-US18984 27 Nov 1996
PRAI US 95-565943 1 Dec 1995
DT Patent
LA English
OS WPI: 97-310355 [28]
AN 97-08966 BIOTECHDS
AB A method for immunizing an individual to prevent disease caused by a **Gram-negative** bacterial pathogen is claimed, which involves vaccinating the individual with a formulation (claimed) consisting of a **Gram-negative** bacterium **htrB mutant**, endotoxin isolated from the **mutant**, endotoxin isolated from the **mutant** and conjugated with a carrier protein, or a **mutant** which has been genetically engineered to express at least one heterologous vaccine antigen as the active ingredient. Also claimed are methods for producing a **mutant** endotoxin or a **Gram-negative** bacterium **mutant** having substantially reduced toxicity as compared with the wild-type endotoxin or bacterium, which involves **mutating** an **htrB** gene within the bacterium causing a phenotype characterized by a **mutant** endotoxin lacking at least one secondary acyl chain on lipid-A contained in the wild-type bacterium. The endotoxins have reduced toxicity compared with the wild-type endotoxins and yet retain antigenicity. The compositions can be used as prophylactic or therapeutic vaccines against endotoxic shock and **Gram-negative** bacteremia. (79pp)

L5 ANSWER 5 OF 21 MEDLINE DUPLICATE 5
AN 97256743 MEDLINE
DN 97256743
TI Function of the Escherichia **coli** msbB gene, a multicopy suppressor of htrB knockouts, in the acylation of lipid A. Acylation by MsbB follows laurate incorporation by HtrB.
AU Clementz T; Zhou Z; Raetz C R
CS Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA.

NC GM-51310 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Apr 18) 272 (16) 10353-60.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199707
EW 19970703
AB Overexpression of the Escherichia **coli** msbB gene on high copy plasmids suppresses the temperature-sensitive growth associated with **mutations** in the **htrB** gene. **htrB** encodes the lauroyl transferase of lipid A biosynthesis that acylates the intermediate (Kdo)2-lipid IVA (Brozek, K. A., and Raetz, C. R. H. (1990) J. Biol. Chem. 265, 15410-15417). Since msbB displays 27.5% identity and 42.2% similarity to **htrB**, we explored the possibility that msbB encodes a related acyltransferase. In contrast to **htrB**, extracts of strains with insertion **mutations** in msbB are not defective in transferring laurate from lauroyl acyl carrier protein to (Kdo)2-lipid IVA. However, extracts of msbB **mutants** do not efficiently acylate the product formed by **HtrB**, designated (Kdo)2-(lauroyl)-lipid IVA. Extracts of strains harboring msbB+ bearing plasmids acylate (Kdo)2-(lauroyl)-lipid IVA very rapidly compared with wild type. We solubilized and partially purified MsbB from an overproducing strain, lacking **HtrB**. MsbB transfers myristate or laurate, activated on ACP, to (Kdo)2-(lauroyl)-lipid IVA. Decanoyl, palmitoyl, palmitoleoyl, and (R)-3-hydroxymyristoyl-ACP are poor acyl donors. MsbB acylates (Kdo)2-(lauroyl)-lipid IVA about 100 times faster than (Kdo)2-lipid IVA. The slow, but measurable, rate whereby MsbB acts on (Kdo)2-lipid IVA may explain why overexpression of MsbB suppresses the temperature-sensitive phenotype of **htrB mutations**. Presumably, the acyloxyacyl group generated by excess MsbB substitutes for the one normally formed by **HtrB**.

L5 ANSWER 6 OF 21 MEDLINE DUPLICATE 6
AN 97431504 MEDLINE
DN 97431504
TI **Mutation** of the **htrB** gene in a virulent **Salmonella** typhimurium strain by intergeneric transduction: strain construction and phenotypic characterization.
AU Sunshine M G; Gibson B W; Engstrom J J; Nichols W A; Jones B D; Apicella M A
CS Department of Microbiology, The University of Iowa, Iowa City 52242, USA.
NC AI38268 (NIAID)
AI24616 (NIAID)
AI31254 (NIAID)

+
SO JOURNAL OF BACTERIOLOGY, (1997 Sep) 179 (17) 5521-33.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199712
AB The **htrB** gene product of **Haemophilus** influenzae contributes to the toxicity of the lipooligosaccharide. The **htrB** gene encodes a 2-keto-3-deoxyoctulosonic acid-dependent acyltransferase which is responsible for myristic acid substitutions at the hydroxy moiety of lipid A beta-hydroxymyristic acid. Mass spectroscopic analysis has demonstrated that lipid A from an H. influenzae **htrB mutant** is predominantly tetraacyl and similar in structure to lipid IV(A), which has been shown to be nontoxic in animal models. We sought to construct a **Salmonella** typhimurium **htrB mutant** in order to investigate the contribution of **htrB** to virulence in a well-defined murine typhoid model of animal pathogenesis. To this end, an r- m+ galE mutS recD strain of S. typhimurium was constructed (MGS-7) and used in inter- and intrastrain transduction experiments with both coliphage P1 and **Salmonella** phage P22. The Escherichia **coli htrB** gene containing a mini-Tn10 insertion was transduced from E. **coli** MLK217 into S. typhimurium MGS-7 via phage P1 and subsequently via phage P22 into the virulent **Salmonella** strain SL1344. All S. typhimurium transductants showed phenotypes similar to those described for the E. **coli htrB mutant**. Mass spectrometric analysis of the crude lipid A fraction from the lipopolysaccharide of the S. typhimurium **htrB mutant** strain showed that for the dominant hexaacyl form, a lauric acid moiety was lost at one position on the lipid A and a palmitic acid moiety was added at another position; for the less abundant heptaacyl species, the lauric acid was replaced with palmitoleic acid.

L5 ANSWER 7 OF 21 MEDLINE DUPLICATE 7
AN 1998013113 MEDLINE
DN 98013113
TI Study of the role of the htrB gene in **Salmonella** typhimurium virulence.
AU Jones B D; Nichols W A; Gibson B W; Sunshine M G; Apicella M A
CS Department of Microbiology, University of Iowa College of Medicine, Iowa City 52242-1109, USA.. bjones@blue.weeg.uiowa.edu
NC AI38268 (NIAID)
RR01614 (NCRR)
AI 31254 (NIAID)
+

SO INFECTION AND IMMUNITY, (1997 Nov) 65 (11) 4778-83.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199801
EW 19980104
AB We have undertaken a study to investigate the contribution of the **htrB** gene to the virulence of pathogenic **Salmonella** typhimurium. An **htrB**::mini-Tn10 **mutation** from Escherichia **coli** was transferred by transduction to the mouse-virulent strain S. typhimurium SL1344 to create an **htrB mutant**. The S. typhimurium **htrB mutant** was inoculated into mice and found to be severely limited in its ability to colonize organs of the lymphatic system and to cause systemic disease in mice. A variety of experiments were performed to determine the possible reasons for this loss of virulence. Serum killing assays revealed that the S. typhimurium **htrB mutant** was as resistant to killing by complement as the wild-type strain. However, macrophage survival assays revealed that the S. typhimurium **htrB mutant** was more sensitive to the intracellular environment of murine macrophages than the wild-type strain. In addition, the bioactivity of the lipopolysaccharide (LPS) of the **htrB mutant** was reduced compared to that of the LPS from the parent strain as measured by both a Limulus amoebocyte lysate endotoxin quantitation assay and a tumor necrosis factor alpha bioassay. These results indicate that the **htrB** gene plays a role in the virulence of S. typhimurium.

L5 ANSWER 8 OF 21 MEDLINE DUPLICATE 8
AN 1998013065 MEDLINE
DN 98013065
TI Evaluation of the virulence of nontypeable **Haemophilus** influenzae lipooligosaccharide **htrB** and rfaD **mutants** in the chinchilla model of otitis media.
AU DeMaria T F; Apicella M A; Nichols W A; Leake E R
CS The Ohio State University, Columbus 43210, USA..
tdemaria@pop.service.acs.ohiostate.edu
NC 5 R01 DC00090-24 (NIDCD)
R01 A124616-08
SO INFECTION AND IMMUNITY, (1997 Nov) 65 (11) 4431-5.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199801

EW 19980104
AB Considerable evidence has implicated nontypeable **Haemophilus** influenzae (NTHi) lipooligosaccharide (LOS) in the pathogenesis of otitis media (OM); however, its exact role has not been conclusively established. Recently, two NTHi LOS-deficient **mutants** have been created and described. Strain 2019-DK1, an rfaD gene **mutant**, expresses a truncated LOS consisting of only three deoxy-D-manno-octulosonic acid residues, a single heptose, and lipid A. Strain 2019-B29, an isogenic **htrB mutant**, possesses an altered oligosaccharide core and an altered lipid A. Each strain's ability to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation was evaluated in the chinchilla model. Nasopharyngeal colonization data indicate that the parent strain and both **mutants** are able to colonize the nasopharynx and exhibit comparable clearance kinetics. Compared with the parent and each other, however, the **mutants** demonstrated marked differences in virulence regarding their relative abilities to induce OM and persist in the middle ear post-transbullar inoculation. Strain B29 required a 3-log-greater dose to induce OM than the parent strain and did not exhibit evidence of sustained multiplication but persisted for the same duration as the parent. Conversely, strain-DK1, even when inoculated at a dose 4 logs greater than the parent dose, was eliminated from the middle ear 72 h after challenge. A comparison of the relative pathogenicities of these isolates provides the opportunity to address fundamental questions regarding the contribution of LOS to pathogenesis issues at the molecular level. Specifically, the impact of these LOS gene disruptions on OM pathogenesis can be defined and may thus provide potential new targets for future protection and intervention strategies.

L5 ANSWER 9 OF 21 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 9
AN 1997:440316 BIOSIS
DN PREV199799739519
TI HtrB of **Haemophilus** influenzae: Determination of biochemical activity and effects on virulence and lipooligosaccharide toxicity.
AU Nichols, W. A.; Raetz, C. R. H.; Clementz, T.; Smith, A. L.; Hanson, J. A.; Ketterer, M. R.; Sunshine, M.; Apicella, M. A. (1)
CS (1) Dep. Microbiol. BSB-3-403, Univ. Iowa Coll. Med., 51 Newton Road, Iowa City, IA 522442 USA
SO Journal of Endotoxin Research, (1997) Vol. 4, No. 3, pp. 163-172. ISSN: 0968-0519.
DT Article
LA English
AB The **htrB mutant** of **Haemophilus** influenzae (strain B29) has been shown to lack secondary (nonhydroxylated) acyl groups in its lipid A. We have determined through in vitro biochemical assays that the **HtrB** protein

acts as a specific acyltransferase in the late stages of lipid A biosynthesis and that the preferred acyl group donor is myristoyl-acyl carrier protein. Under the conditions employed, the Escherichia **coli** precursor, Kdo-2-lipid IV-A, functions as a myristate acceptor. Introduction of the **Haemophilus htrB** gene into an E. **coli mutant** lacking **htrB** complements the biochemical and physiological defects associated with the E. **coli htrB mutation**. Tumor necrosis factor alpha (TNF-alpha) assays using murine and human macrophage cells indicated that nontypeable H. influenzae (NtHi) strain 2019 and H. influenzae type b strain A2 elicit levels of expression of TNF-alpha that are 30-40 times greater than levels induced by the isogenic **htrB mutants** (B29 and A2B29). Studies using cell-free LOS indicated that the LOS from wild type strain 2019 elicits levels of TNF-alpha expression that are 6-8-fold higher than those of B29. In situ hybridization studies of a primary human bronchial epithelial cell line demonstrated a greater increase of TNF-alpha message produced in the presence of 2019 LOS than in the presence of B29 LOS. TNF-alpha levels of the cell supernatant of cells stimulated with 2019 LOS were found to be 7-8-fold higher than levels in B29 stimulated supernatants. Using the Limulus amoebocyte lysate for assessment of endotoxic activity, we found that wild type LOS was 8-fold higher in endotoxic activity compared with the **mutant** LOS. In virulence assays using intraperitoneal inoculation of infant rats, the **htrB** isogenic strain caused bacteremia at 50% the frequency of the wild type strain. In intranasal inoculation studies, the **htrB mutant** strain was unable to cause bacteremia whereas the wild type b parent produced bacteremia in 40-60% of the animals. These findings suggest that the **htrB** gene of H. influenzae is important for virulence and that host TNF-alpha expression is attenuated in response to **htrB mutant** strains.

L5 ANSWER 10 OF 21 MEDLINE DUPLICATE 10
AN 96405645 MEDLINE
DN 96405645
TI Mutational analysis and properties of the msbA gene of Escherichia **coli**, coding for an essential ABC family transporter.
AU Polissi A; Georgopoulos C
CS Department de Biochimie Medicale, Centre Medical Universitaire, Geneva, Switzerland.. AP44783@ggr.co.uk
SO MOLECULAR MICROBIOLOGY, (1996 Jun) 20 (6) 1221-33.
Journal code: MOM. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199704

EW 19970404

AB The **htrB** gene was discovered because its insertional inactivation interfered with Escherichia **coli** growth and viability at temperatures above 32.5 degrees C, as a result of accumulation of phospholipids. The msbA gene was originally discovered because when cloned on a low-copy-number plasmid vector it was able to suppress the temperature-sensitive growth phenotype of an **htrB** null **mutant** as well as the accumulation of phospholipids. The msbA gene product belongs to the superfamily of ABC transporters, a universally conserved family of proteins characterized by a highly conserved ATP-binding domain. The msbA gene is essential for bacterial viability at all temperatures. In order to understand the physiological role of the MsbA protein, we **mutated** the ATP-binding domain using random PCR **mutagenesis**. Six independent **mutants** were isolated and characterized. Four of these **mutations** resulted in single-amino-acid substitutions in non-conserved residues and were able to support cell growth at 30 degrees C but not at 43 degrees C. The remaining two **mutations** behaved as recessive lethals, and resulted in single-amino-acid substitutions in Walker motif B, one of the two highly conserved regions of the ATP-binding domain. Despite the fact that neither of these two **mutant** proteins can support E. **coli** growth, they both retained the ability to bind ATP in vitro. In addition, we present evidence to show that N-acetyl [3H]-glucosamine, a precursor of lipopolysaccharides, accumulates at the non-permissive temperature in the inner membrane of either **htrB** null or msbA conditional lethal strains. Translocation of the precursor to the outer membrane is restored by transformation with a plasmid containing the wild-type msbA gene. A possible role for MsbA as a translocator of lipopolysaccharides or its precursors is discussed.

L5 ANSWER 11 OF 21 BIOSIS COPYRIGHT 1999 BIOSIS

AN 1996:259716 BIOSIS

DN PREV199698815845

TI **Haemophilus** influenzae **htrB mutants** induce a reduced production of tumor necrosis factor by mouse macrophage-like cells.

AU Nichols, Wade A. (1); Sunshine, Melvin G. (1); Harty, John T. (1); Smith, Arnold L.; Apicella, Michael A. (1)

CS (1) Univ. Iowa, Iowa City, IA USA

SO Abstracts of the General Meeting of the American Society for Microbiology, (1996) Vol. 96, No. 0, pp. 232.
Meeting Info.: 96th General Meeting of the American Society for Microbiology New Orleans, Louisiana, USA May 19-23, 1996
ISSN: 1060-2011.

DT Conference

LA English

L5 ANSWER 12 OF 21 MEDLINE DUPLICATE 11
AN 96070820 MEDLINE
DN 96070820
TI **Mutation** of the **htrB** locus of **Haemophilus** influenzae nontypable strain 2019 is associated with modifications of lipid A and phosphorylation of the lipo-oligosaccharide.
AU Lee N G; Sunshine M G; Engstrom J J; Gibson B W; Apicella M A
CS Department of Microbiology, University of Iowa, Iowa City 52242, USA..
NC AI 24616 (NIAID)
NCRR BRTP 01614
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 10) 270 (45) 27151-9. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U17642
EM 199602
AB The **HtrB** protein was first identified in Escherichia **coli** as a protein required for cell viability at high temperature, but its expression was not regulated by temperature. We isolated an **htrB** homologue from non-typable **Haemophilus** influenzae strain (NTHi) 2019, which was able to functionally complement the E. **coli htrB mutation**. The promoter for the NTHi 2019 **htrB** gene overlaps the promoter for the rfaE gene, and the two genes are divergently transcribed. The deduced amino acid sequence of NTHi 2019 **HtrB** had 56% homology to E. **coli HtrB**. In vitro transcription-translation analysis confirmed production of a protein with an apparent molecular mass of 32-33 kDa. Primer extension analysis revealed that **htrB** was transcribed from a sigma 70-dependent consensus promoter and its expression was not affected by temperature. The expression of **htrB** and rfaE was 2.5-4 times higher in the NTHi **htrB mutant** B29 than in the parental strain. In order to study the function of the **HtrB** protein in **Haemophilus**, we generated two isogenic **htrB mutants** by shuttle **mutagenesis** using a mini-Tn3. The **htrB mutants** initially showed temperature sensitivity, but they lost the sensitivity after a few passages at 30 degrees C and were able to grow at 37 degrees C. They also showed hypersensitivity to deoxycholate and kanamycin, which persisted on passage. SDS-polyacrylamide gel electrophoresis analysis revealed that the lipo-oligosaccharide (LOS) isolated from these **mutants** migrated faster than the wild type LOS and its color changed from black to brown as has been described for E. **coli htrB mutants**. Immunoblotting

analysis also showed that the LOS from the **htrB mutants** lost reactivity to a monoclonal antibody, 6E4, which binds to the wild type NTHi 2019 LOS. Electrospray ionization-mass spectrometry analysis of the O-deacylated LOS oligosaccharide indicated a modification of the core structure characterized in part by a net loss in phosphoethanolamine. Mass spectrometric analysis of the lipid A of the **htrB mutant** indicated a loss of one or both myristic acid substitutions. These data suggest that **HtrB** is a multifunctional protein and may play a controlling role in regulating cell responses to various environmental changes.

L5 ANSWER 13 OF 21 MEDLINE DUPLICATE 12
AN 95172727 MEDLINE
DN 95172727
TI Molecular cloning and characterization of the nontypeable **Haemophilus** influenzae 2019 rfaE gene required for lipopolysaccharide biosynthesis.
AU Lee N G; Sunshine M G; Apicella M A
CS Department of Microbiology, University of Iowa, Iowa City 52242..
NC AI 24616 (NIAID)
SO INFECTION AND IMMUNITY, (1995 Mar) 63 (3) 818-24.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U17642
EM 199506
AB The lipooligosaccharide (LOS) of nontypeable **Haemophilus** influenzae (NTHi) is an important factor in pathogenesis and virulence. In an attempt to elucidate the genes involved in LOS biosynthesis, we have cloned the rfaE gene from NTHi 2019 by complementing a **Salmonella** typhimurium rfaE **mutant** strain with an NTHi 2019 plasmid library. The rfaE **mutant** synthesizes lipopolysaccharide (LPS) lacking heptose, and the rfaE gene is postulated to be involved in ADP-heptose synthesis. Retransformation with the plasmid containing 4 kb of NTHi DNA isolated from a reconstituted **mutant** into rfaE **mutants** gave wild-type LPS phenotypes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis confirmed the conversion of the rfaE **mutant** LPS to a wild-type LPS phenotype. Sequence analysis of a 2.4-kb BglII fragment revealed two open reading frames. One open reading frame encodes the RfaE protein with a molecular weight of 37.6 kDa, which was confirmed by in vitro transcription and translation, and the other encodes a polypeptide highly homologous to the Escherichia **coli HtrB** protein. These two genes are transcribed from the same promoter region into opposite directions. Primer extension analysis of the rfaE gene revealed a single transcription start site at 37 bp

upstream of the predicted translation start site. The upstream promoter region contained a sequence (TA AAAT) homologous to the -10 region of the bacterial sigma 70-dependent promoters at an appropriate distance (7 bp), but not sequence resembling the consensus sequence of the -35 region was found. These studies demonstrate the ability to use complementation of defined LPS defects in members of the family Enterobacteriaceae to identify LOS synthesis genes in NTHi.

L5 ANSWER 14 OF 21 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1995:290502 BIOSIS
DN PREV199598304802
TI Isolation and **mutant** analysis of the **htrb** homologue of the **Haemophilus** influenzae nontypable strain 2019.
AU Lee, Na-Gyong; Sunshine, Melvin G.; Engstrom, Jeffrey; Gibson, Bradford W.; Apicella, Michael A.
CS University Iowa, Iowa City, IA USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 206.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DT Conference
LA English

L5 ANSWER 15 OF 21 MEDLINE DUPLICATE 13
AN 93172962 MEDLINE
DN 93172962
TI The essential Escherichia **coli** msbA gene, a multicopy suppressor of null **mutations** in the **htrB** gene, is related to the universally conserved family of ATP-dependent translocators.
AU Karow M; Georgopoulos C
CS Department of Cellular, Viral and Molecular Biology, School of Medicine, University of Utah, Salt Lake City 84132.
NC AI21029 (NIAID)
GM07464 (NIGMS)
SO MOLECULAR MICROBIOLOGY, (1993 Jan) 7 (1) 69-79.
Journal code: MOM. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-Z11796
EM 199305
AB We report the characterization of the msbA gene, isolated as a multicopy suppressor of the **HtrB** temperature-sensitive phenotype. The msbA gene maps to 20.5 min on the Escherichia

coli genetic map and encodes a protein with an estimated molecular mass of 64,460 Da, with the properties of an integral membrane protein. The amino acid sequence of MsbA is very similar to those of the family of ATP-dependent translocators, which includes the haemolysin B protein of E. **coli** and the mammalian multidrug resistance (MDR) proteins. **Mutational** analysis of msbA indicates that it may form an operon with a downstream gene, orfE, and that both of these genes are essential for bacterial viability under all growth conditions tested.

L5 ANSWER 16 OF 21 MEDLINE DUPLICATE 14
AN 93054357 MEDLINE
DN 93054357
TI The lethal phenotype caused by null **mutations** in the Escherichia **coli htrB** gene is suppressed by **mutations** in the accBC operon, encoding two subunits of acetyl coenzyme A carboxylase.
AU Karow M; Fayet O; Georgopoulos C
CS Department of Cellular, Viral, and Molecular Biology, School of Medicine, University of Utah, Salt Lake City 84132.
NC HL34127 (NHLBI)
AI21029 (NIAID)
GM07464 (NIGMS)
SO JOURNAL OF BACTERIOLOGY, (1992 Nov) 174 (22) 7407-18.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199302
AB Insertion **mutations** in the Escherichia **coli htrB** gene result in the unique phenotype of not affecting growth at temperatures below 32.5 degrees C but leading to a loss of viability at temperatures above this in rich media. When **htrB** bacteria growing in rich media were shifted to the nonpermissive temperature of 42 degrees C, they continued to grow at a rate similar to that at 30 degrees C but they produced phospholipids at the rate required for growth at 42 degrees C. This led to the accumulation of more than twice as much phospholipid per milligram of protein compared with that in wild-type bacteria. Consistent with **HtrB** playing a role in phospholipid biosynthesis, one complementation group of spontaneously arising **mutations** that suppressed **htrB**-induced lethality were mapped to the accBC operon. This operon codes for the biotin carboxyl carrier protein and biotin carboxylase subunits of the acetyl coenzyme A carboxylase enzyme complex, which catalyzes the first step in fatty acid biosynthesis. Four suppressor **mutations** mapped to this operon. Two alleles were identified as **mutations** in the accC gene, the third allele was

identified as a **mutation** in the accB gene, and the fourth allele was shown to be an insertion of an IS1 transposable element in the promoter region of the operon, resulting in reduced transcription. The suppressor **mutations** caused a decrease in the rate of phospholipid biosynthesis, restoring the balance between the biosynthesis of phospholipids and growth rate, thus enabling **htrB** bacteria to grow at high temperatures.

L5 ANSWER 17 OF 21 MEDLINE DUPLICATE 15
AN 92121107 MEDLINE
DN 92121107
TI Isolation and characterization of the Escherichia **coli** msbB gene, a multicopy suppressor of null **mutations** in the high-temperature requirement gene **htrB**.
AU Karow M; Georgopoulos C
CS Department of Cellular, Viral and Molecular Biology, School of Medicine, University of Utah, Salt Lake City 84132.
NC AI21029 (NIAID)
GM07464 (NIGMS)
SO JOURNAL OF BACTERIOLOGY, (1992 Feb) 174 (3) 702-10.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-M77039; GENBANK-Z11767; GENBANK-Z11768; GENBANK-L01135; GENBANK-L01136; GENBANK-L01137; GENBANK-L01138; GENBANK-L01139; GENBANK-L01140; GENBANK-L01141
EM 199204
AB Previous work established that the **htrB** gene of Escherichia **coli** is required for growth in rich media at temperatures above 32.5 degrees C but not at lower temperatures. In an effort to determine the functional role of the **htrB** gene product, we have isolated a multicopy suppressor of **htrB**, called msbB. The msbB gene has been mapped to 40.5 min on the E. **coli** genetic map, in a 12- to 15-kb gap of the genomic library made by Kohara et al. (Y. Kohara, K. Akiyama, and K. Isono, Cell 50:495-508, 1987). Mapping data show that the order of genes in the region is eda-edd-zwf-pykA-msbB. The msbB gene codes for a protein of 37,410 Da whose amino acid sequence is similar to that of **HtrB** and, like **HtrB**, the protein is very basic in nature. The similarity of the **HtrB** and MsbB proteins could indicate that they play functionally similar roles. **Mutational** analysis of msbB shows that the gene is not essential for E. **coli** growth; however, the **htrB** msbB double **mutant** exhibits a unique morphological phenotype at 30 degrees C not seen with either of the single **mutants**. Analysis of both msbB and **htrB** **mutants** shows that these bacteria are resistant to four

times more deoxycholate than wild-type bacteria but not to other hydrophobic substances. The addition of quaternary ammonium compounds rescues the temperature-sensitive phenotype of **htrB** bacteria, and this rescue is abolished by the simultaneous addition of Mg2+ or Ca2+. These results suggest that MsbB and **HtrB** play an important role in outer membrane structure and/or function.

L5 ANSWER 18 OF 21 MEDLINE DUPLICATE 16
AN 92114808 MEDLINE
DN 92114808
TI Sequencing, **mutational** analysis, and transcriptional regulation of the Escherichia **coli htrB** gene.
AU Karow M; Georgopoulos C
CS Department of Cellular, Viral, and Molecular Biology, School of Medicine, University of Utah, Salt Lake City 84132..
NC AI21029 (NIAID)
GM07464 (NIGMS)
SO MOLECULAR MICROBIOLOGY, (1991 Sep) 5 (9) 2285-92.
Journal code: MOM. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X61000; GENBANK-S76453; GENBANK-X61210; GENBANK-S77306; GENBANK-S77307; GENBANK-X59272; GENBANK-X52368; GENBANK-X52369; GENBANK-X52370; GENBANK-X52371; GENBANK-X52372
EM 199204
AB The Escherichia **coli** htrB gene was originally discovered because its insertional inactivation led to an exquisitely temperature-sensitive phenotype in rich media, i.e. the ability to form colonies at temperatures below 32 degrees C, but not above 33 degrees C. The htrB gene has been sequenced. It can potentially code for two proteins, with Mr values of 35,407 Da and 8669 Da, that are encoded by overlapping, divergent open reading frames. Our data are consistent with the 35,407 Da protein being HtrB. Northern blot analysis clearly shows that the monocistronic htrB message is not under heat-shock regulation. We have also sequenced the flanking DNA and have discovered a new gene, designated orf39.9, located immediately adjacent to htrB, but divergently transcribed.

L5 ANSWER 19 OF 21 MEDLINE DUPLICATE 17
AN 91100364 MEDLINE
DN 91100364
TI Isolation and characterization of the Escherichia **coli** htrB gene, whose product is essential for bacterial viability above 33 degrees C in rich media.
AU Karow M; Fayet O; Cegielska A; Ziegelhoffer T; Georgopoulos C
CS Department of Cellular, Viral, and Molecular Biology, University of

Utah School of Medicine, Salt Lake City 84132.
NC AI21029 (NIAID)
GM07464 (NIGMS)
SO JOURNAL OF BACTERIOLOGY, (1991 Jan) 173 (2) 741-50.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199104
AB We have identified and studied the **htrB** gene of Escherichia **coli**. Insertional inactivation of the **htrB** gene leads to bacterial death at temperatures above 33 degrees C. The **mutant** bacterial phenotype at nonpermissive temperatures includes an arrest of cell division followed by the formation of bulges or filaments. The **htrB**+ gene has been cloned by complementation and shown to reside at 23.4 min on the E. **coli** genetic map, the relative order of the neighboring loci being mboA-**htrB**-pyrC. The **htrB** gene is transcribed in a counterclockwise fashion, relative to the E. **coli** genetic map, and its product has been identified as a membrane-associated protein of 35,000 Da. Growth experiments in minimal media indicate that the **HtrB** function becomes dispensable at low growth rates.

L5 ANSWER 20 OF 21 MEDLINE DUPLICATE 18
AN 92021792 MEDLINE
DN 92021792
TI Complex phenotypes of null mutations in the htr genes, whose products are essential for Escherichia **coli** growth at elevated temperatures.
AU Karow M; Raina S; Georgopoulos C; Fayet O
CS Department of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City 84132.
NC AI21029 (NIAID)
GM07464 (NIGMS)
SO RESEARCH IN MICROBIOLOGY, (1991 Feb-Apr) 142 (2-3) 289-94.
Journal code: R6F. ISSN: 0923-2508.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199201
AB Transposon insertion, followed by screening, has allowed the identification of a set of genes, called htr, whose products are required for Escherichia **coli** growth at elevated temperatures. The **htrB** gene has been shown to map at 23.5 min on the E. **coli** genetic map. It codes for a very basic, hydrophobic, 35,000-Mr polypeptide, possessing a putative

membrane-spanning domain. At the non-permissive temperature, **htrB mutant** bacteria stop dividing, followed by the formation of bulges and eventual lysis. The htrC gene maps at 90 min, is under sigma 32 regulation and codes for a 21, 130-Mr polypeptide. At 43 degrees C, htrC **mutant** bacteria gradually lyse, whereas at intermediate temperatures they filament extensively. Finally, the htrM gene maps at 81 min, is under sigma 32 regulation and codes for a 35,000-Mr polypeptide. The HtrM null phenotype included inability to grow above 42 degrees C, extreme mucoidness and sensitivity to bile salts, even at the permissive temperatures. The htrM gene is identical to the rfaD gene, whose product is required for the biosynthesis of the lipopolysaccharide precursor ADP-L-glycero-D-mannoheptose (Pegues et al., J. Bact., 1990, 172, 4652-4660).

L5 ANSWER 21 OF 21 LIFESCI COPYRIGHT 1999 CSA
AN 91:45067 LIFESCI
TI Complex phenotypes of null mutations in the htr genes, whose products are essential for Escherichia **coli** growth at elevated temperatures.
THE BACTERIAL CELL CYCLE: STRUCTURAL AND MOLECULAR ASPECTS.
AU Karow, M.; Raina, S.; Georgopoulos, C.; Fayet, O.; Bouche, J.-P. [editor]; D'Ari, R. [editor]; Louarn, J.-M. [editor]
CS Dep. Cell., Viral and Mol. Biol., Univ. Utah Med. Cent., Salt Lake City, UT 84132, USA
SO RES. MICROBIOL., (1991) pp. 289-294.
Meeting Info.: EMBO Workshop on the Bacterial Cell Cycle: Structural and Molecular Aspects. Collonges-La-Rouge (France). 1-4 Oct 1990.
DT Book
TC Conference
FS J; G
LA English
SL English
AB Transposon insertion, followed by screening, has allowed the identification of a set of genes, called htr, whose products are required for Escherichia **coli** growth at elevated temperatures. The **htrB** gene has been shown to map at 23.5 min on the E. **coli** genetic map. It codes for a very basic, hydrophobic, 35,000-Mr polypeptide, possessing a putative membrane-spanning domain. At the non-permissive temperature, **htrB mutant** bacteria stop dividing, followed by the formation of bulges and eventual lysis. The htrC gene maps at 90 min, is under sigma super(32) regulation and codes for a 21,130-Mr polypeptide. The htrM gene is identical to the rfaD gene, whose product is required for the biosynthesis of the lipopolysaccharide precursor ADP-L-glycero-D mannoheptose.

=> d his 16-; d 1-10 bib abs

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, BIOTECHDS, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, DRUGU, DRUGNL, DRUGB' ENTERED AT 10:32:59 ON 01 MAR 1999)

Author (s)

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L6          969 S APICELLA M?/AU
L7          210 S SUNSHINE M?/AU
L8         6316 S LEE N?/AU
L9          144 S ARUMUGHAM R?/AU
L10        2123 S GIBSON B?/AU
L11           3 S L6 AND L7 AND L8 AND L9 AND L10
L12         164 S L6 AND (L7 OR L8 OR L9 OR L10)
L13          40 S L7 AND (L8 OR L9 OR L10)
L14          17 S L8 AND (L9 OR L10)
L15           3 S L9 AND L10
L16        9538 S L6 OR L7 OR L8 OR L9 OR L10
L17          45 S (L12 OR L13 OR L16) AND L1
L18          52 S L11 OR L14 OR L15 OR L17
L19          10 DUP REM L18 (42 DUPLICATES REMOVED)
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L19 ANSWER 1 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1

AN 1997:496805 CAPLUS

DN 127:107983

TI Non-toxic mutants of pathogenic gram-negative bacteria

IN **Apicella, Michael A.; Sunshine, Melvin G.; Lee, Na-gyong; Arumugham, Rasappa; Gibson, Bradford W.**

PA University of Iowa Research Foundation, USA; The Regents of the University of California; American Cyanamid Company; Apicella, Michael A.; Sunshine, Melvin G.; Lee, Na-Gyong; Arumugham, Rasappa; Gibson, Bradford W.

SO PCT Int. Appl., 78 pp.
CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9719688	A1	19970605	WO 96-US18984	19961127
	W: AU, CA, JP, KR, MX, NZ, US, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	CA 2238640	AA	19970605	CA 96-2238640	19961127
	AU 9711246	A1	19970619	AU 97-11246	19961127
	EP 876150	A1	19981111	EP 96-942080	19961127
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRAI US 95-565943 19951201
WO 96-US18984 19961127

AB A method is provided for identifying, isolating, and producing

htrB mutants of gram-neg. bacterial pathogens. The method comprises **mutating** the **htrB** gene of a gram-neg. bacterial pathogen so that there is a lack of a functional **htrB** protein, resulting in a **mutant** that lacks .gtoreq.1 secondary acyl chains contained in the wild type gram-neg. bacterial pathogen, and displays substantially reduced toxicity as compared to the wild type strain. The present invention also provides methods for using a vaccine formulation contg. the **htrB mutant**, the endotoxin isolated therefrom, or the endotoxin isolated therefrom which is then conjugated to a carrier protein to immunize an individual against infections caused by gram-neg. bacterial pathogens by administering a prophylactically effective amt. of the vaccine formulation.

L19 ANSWER 2 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2
AN 1997:591347 CAPLUS
DN 127:288753
TI **Mutation** of the **htrB** gene in a virulent Salmonella typhimurium strain by intergeneric transduction: strain construction and phenotypic characterization
AU **Sunshine, Melvin G.**; **Gibson, Bradford W.**; Engstrom, Jeffrey J.; Nichols, Wade A.; Jones, Bradley D.; **Apicella, Michael A.**
CS Department Microbiology, University Iowa, Iowa City, IA, 52242, USA
SO J. Bacteriol. (1997), 179(17), 5521-5533
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB The htrB gene product of Haemophilus influenzae contributes to the toxicity of the lipooligosaccharide. The htrB gene encodes a 2-keto-3-deoxyoctulosonic acid-dependent acyltransferase which is responsible for myristic acid substitutions at the hydroxy moiety of lipid A .beta.-hydroxymyristic acid. Mass spectroscopic anal. has demonstrated that lipid A from an H. influenzae **htrB mutant** is predominantly tetraacyl and similar in structure to lipid IVA, which has been shown to be nontoxic in animal models. We sought to construct a Salmonella typhimurium **htrB mutant** in order to investigate the contribution of **htrB** to virulence in a well-defined murine typhoid model of animal pathogenesis. To this end, an r- m+ galE mutS recD strain of S. typhimurium was constructed (MGS-7) and used in inter- and intrastrain transduction expts. with both coliphage P1 and Salmonella phage P22. The Escherichia coli htrB gene contg. a mini-Tn10 insertion was transduced from E. coli MLK217 into S. typhimurium MGS-7 via phage P1 and subsequently via phage P22 into the virulent Salmonella strain SL1344. All S. typhimurium transductants showed phenotypes similar to those described for the E. coli **htrB mutant**. Mass spectrometric anal.

of the crude lipid A fraction from the lipopolysaccharide of the S. typhimurium **htrB mutant** strain showed that for the dominant hexaacyl form, a lauric acid moiety was lost at one position on the lipid A and a palmitic acid moiety was added at another position; for the less abundant heptaacyl species, the lauric acid was replaced with palmitoleic acid.

L19 ANSWER 3 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
AN 1997:731002 CAPLUS
DN 128:20429
TI Study of the role of the htrB gene in Salmonella typhimurium virulence
AU Jones, Bradely D.; Nichols, Wade A.; **Gibson, Bradford W.**; **Sunshine, Melvin G.**; **Apicella, Michael A.**
CS Dep. Microbiology, Univ. Iowa College Medicine, Iowa City, IA, 52242-1109, USA
SO Infect. Immun. (1997), 65(11), 4778-4783
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB We have undertaken a study to investigate the contribution of the htrB gene to the virulence of pathogenic Salmonella typhimurium. An **htrB**::mini-Tn10 **mutation** from Escherichia coli was transferred by transduction to the mouse-virulent strain S. typhimurium SL1344 to create an **htrB mutant**. The S. typhimurium **htrB mutant** was inoculated into mice and found to be severely limited in its ability to colonize organs of the lymphatic system and to cause systemic disease in mice. A variety of expts. were performed to det. the possible reasons for this loss of virulence. Serum killing assays revealed that the S. typhimurium **htrB mutant** was as resistant to killing by complement as the wild-type strain. However, macrophage survival assays revealed that the S. typhimurium **htrB mutant** was more sensitive to the intracellular environment of murine macrophages than the wild-type strain. In addn., the bioactivity of the lipopolysaccharide (LPS) of the **htrB mutant** was reduced compared to that of the LPS from the parent strain as measured by both a Limulus amoebocyte lysate endotoxin quantitation assay and a tumor necrosis factor alpha bioassay. These results indicated that the htrB gene plays a role in the virulence of S. typhimurium.

L19 ANSWER 4 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4
AN 1997:730328 CAPLUS
DN 128:21296
TI Evaluation of the virulence of nontypeable Hemophilus influenzae lipooligosaccharide **htrB** and rfaD **mutants** in the chinchilla model of otitis media

AU DeMaria, T. F.; **Apicella, M. A.**; Nichols, W. A.; Leake, E. R.
CS Div. of Otologic Research, College of Medicine, Ohio State Univ., Columbus, OH, 43210, USA
SO Infect. Immun. (1997), 65(11), 4431-4435
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB Considerable evidence has implicated nontypeable Hemophilus influenzae (NTHi) lipooligosaccharide (LOS) in the pathogenesis of otitis media (OM); however, its exact role has not been conclusively established. Recently, two NTHi LOS-deficient mutants have been created and described. Strain 2019-DK1, an rfaD gene mutant, expresses a truncated LOS consisting of only three deoxy-D-manno-octulosonic acid residues, a single heptose, and lipid A. Strain 2019-B29, an isogenic **htrB mutant**, possesses an altered oligosaccharide core and an altered lipid A. Each strain's ability to colonize the nasopharynx and to induce OM subsequent to transbullar inoculation was evaluated in the chinchilla model. Nasopharyngeal colonization data indicate that the parent strain and both mutants are able to colonize the nasopharynx and exhibit comparable clearance kinetics. Compared with the parent and each other, however, the mutants demonstrated marked differences in virulence regarding their relative abilities to induce OM and persist in the middle ear post-transbullar inoculation. Strain B29 required a 3-log-greater dose to induce OM than the parent strain and did not exhibit evidence of sustained multiplication but persisted for the same duration as the parent. Conversely, strain-DK1, even when inoculated at a dose 4 logs greater than the parent dose, was eliminated from the middle ear 72 h after challenge. A comparison of the relative pathogenicities of these isolates provides the opportunity to address fundamental questions regarding the contribution of LOS to pathogenesis issues at the mol. level. Specifically, the impact of these LOS gene disruptions on OM pathogenesis can be defined and may thus provide potential new targets for future protection and intervention strategies.

L19 ANSWER 5 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 5
AN 1997:229116 CAPLUS
DN 126:302223
TI Identification of the ADP-L-glycero-D-manno-heptose-6-epimerase (rfaD) and heptosyltransferase II (rfaF) biosynthesis genes from nontypeable Haemophilus influenzae 2019
AU Nichols, Wade A.; **Gibson, Bradford W.**; Melaugh, William; **Lee, Na-Gyong**; Sunshine, Melvin; Apicella, Michael A.
CS Department of Microbiology, University of Iowa College of Medicine, Iowa City, IA, 52242, USA

SO Infect. Immun. (1997), 65(4), 1377-1386
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB Haemophilus influenzae is an important human pathogen. The lipooligosaccharide (LOS) of H. influenzae has been implicated as a virulence determinant. To better understand the assembly of LOS in nontypeable H. influenzae (NtHi), the authors have cloned and characterized the rfaD and rfaF genes of NtHi 2019, which encode the ADP-L-glycero-D-manno-heptose-6-epimerase and heptosyltransferase II enzymes, resp. This cloning was accomplished by the complementation of Salmonella typhimurium lipopolysaccharide (LPS) biosynthesis gene mutants. These deep rough mutants are novobiocin susceptible until complemented with the appropriate gene. In this manner, the authors are able to use novobiocin resistance to select for specific NtHi LOS inner core biosynthesis genes. Such a screening system yielded a plasmid with a 4.8-kb insert. This plasmid was able to complement both rfaD and rfaF mutants of S. typhimurium. The LPS of these complemented strains appeared identical to the wild-type Salmonella LPS. The genes encoding the rfaD and rfaF genes from NtHi 2019 were sequenced and found to be similar to the analogous genes from S. typhimurium and Escherichia coli. The rfaD gene encodes a polypeptide of 35 kDa and the rfaF encodes a protein of 39 kDa, as demonstrated by in vitro transcription-translation studies. Isogenic mutants which demonstrated truncated LOS consistent with inner core biosynthesis mutants were constructed in the NtHi strain 2019. Primer extension anal. demonstrated the presence of a strong promoter upstream of rfaD but suggested only a very weak promoter upstream of rfaF. Complementation studies, however, suggest that the rfaF gene does have an independent promoter. Mass spectrometric anal. shows that the LOS mols. expressed by H. influenzae rfaD and rfaF mutant strains have identical mol. masses. Addnl. studies verified that in the rfaD mutant strain, D-glycero-D-manno-heptose is added to the LOS mol. in place of the usual L-glycero-D-manno-heptose. Finally, the genetic organizations of the inner core biosynthesis genes of S. typhimurium, E. coli, and several strains of H. influenzae were examd., and substantial differences were uncovered.

L19 ANSWER 6 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 6
AN 1997:605036 CAPLUS
DN 127:275201
TI htrB of Haemophilus influenzae: determination of biochemical activity and effects on virulence and lipooligosaccharide toxicity
AU Nichols, W. A.; Raetz, C. R. H.; Clementz, T.; Smith, A. L.; Hanson, J. A.; Ketterer, M. R.; **Sunshine, M.**; **Apicella, M. A.**
CS Department of Microbiology, University of Iowa College of Medicine,

Iowa City, IA, 522442, USA
SO J. Endotoxin Res. (1997), 4(3), 163-172
CODEN: JENREB; ISSN: 0968-0519
PB Churchill Livingstone
DT Journal
LA English
AB The **htrB mutant** of Haemophilus influenzae (strain B29) has been shown to lack secondary (nonhydroxylated) acyl groups in its lipid A. The authors have detd. through in vitro biochem. assays that the HtrB protein acts as a specific acyltransferase in the late stages of lipid A biosynthesis and that the preferred acyl group donor is myristoyl-acyl carrier protein. Under the conditions employed, the Escherichia coli precursor, Kdo2-lipid IVA, functions as a myristate acceptor. Introduction of the Haemophilus **htrB** gene into an E. coli **mutant** lacking **htrB** complements the biochem. and physiol. defects assocd. with the E. coli **htrB mutation**. Tumor necrosis factor .alpha. (TNF.alpha.) assays using murine and human macrophage cells indicated that nontypeable H. influenzae (NtHi) strain 2019 and H. influenzae type b strain A2 elicit levels of expression of TNF.alpha. that are 30-40 times greater than levels induced by the isogenic **htrB mutants** (B29 and A2B29). Studies using cell-free LOS indicated that the LOS from wild type strain 2019 elicits levels of TNF.alpha. expression that are 6-8-fold higher than those of B29. In situ hybridization studies of a primary human bronchial epithelial cell line demonstrated a greater increase of TNF.alpha. message produced in the presence of 2019 LOS than in the presence of B29 LOS. TNF.alpha. levels of the cell supernatant of cells stimulated with 2019 LOS were found to be 7-8-fold higher than levels in B29 stimulated supernatants. Using the Limulus amoebocyte lysate for assessment of endotoxic activity, we found that wild type LOS was 8-fold higher in endotoxic activity compared with the mutant LOS. In virulence assays using i.p. inoculation of infant rats, the htrB isogenic strain caused bacteremia at 50% the frequency of the wild type strain. In intranasal inoculation studies, the **htrB mutant** strain was unable to cause bacteremia whereas the wild type b parent produced bacteremia in 40-60% of the animals. These findings suggest that the **htrB** gene of H. influenzae is important for virulence and that host TNF.alpha. expression is attenuated in response to **htrB mutant** strains.

L19 ANSWER 7 OF 10 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:259716 BIOSIS
DN PREV199698815845
TI Haemophilus influenzae **htrB mutants** induce a reduced production of tumor necrosis factor by mouse macrophage-like cells.
AU Nichols, Wade A. (1); **Sunshine, Melvin G. (1)**; Harty, John

T. (1); Smith, Arnold L.; **Apicella, Michael A. (1)**
CS (1) Univ. Iowa, Iowa City, IA USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1996) Vol. 96, No. 0, pp. 232.
Meeting Info.: 96th General Meeting of the American Society for Microbiology New Orleans, Louisiana, USA May 19-23, 1996
ISSN: 1060-2011.
DT Conference
LA English

L19 ANSWER 8 OF 10 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 7
AN 1995:945651 CAPLUS
DN 124:25327
TI **Mutation** of the **htrB** locus of Haemophilus influenzae nontypable strain 2019 is associated with modifications of lipid A and phosphorylation of the lipo-oligosaccharide
AU **Lee, Na-Gyong; Sunshine, Melvin G.**; Engstrom, Jeffery J.; **Gibson, Bradford W.**; **Apicella, Michael A.**
CS Dep. Microbiol., Univ. Iowa, Iowa City, IA, 52242, USA
SO J. Biol. Chem. (1995), 270(45), 27151-9
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The HtrB protein was first identified in Escherichia coli as a protein required for cell viability at high temp., but its expression was not regulated by temp. An **htrB** homolog was isolated from nontypable Haemophilus influenzae strain (NTHi) 2019, which was able to functionally complement the E. coli **htrB mutation**. The promoter for the NTHi 2019 htrB gene overlaps the promoter for the rfaE gene, and the 2 genes are divergently transcribed. The deduced amino acid sequence of NTHi 2019 HtrB had 56% homol. to E. coli HtrB. In vitro transcription-translation anal. confirmed prodn. of a protein with an apparent mol. mass of 32-33 kDa. Primer extension anal. revealed that htrB was transcribed from a .sigma.70-dependent consensus promoter and its expression was not affected by temp. The expression of **htrB** and rfaE was 2.5-4-fold higher in the NTHi **htrB mutant** B29 than in the parental strain. In order to study the function of the **HtrB** protein in Haemophilus, 2 isogenic **htrB mutants** were generated by shuttle **mutagenesis** using a mini-Tn3. The **htrB mutants** initially showed temp. sensitivity, but they lost the sensitivity after a few passages at 30.degree. and were able to grow at 37.degree.. They also showed hypersensitivity to deoxycholate and kanamycin, which persisted on passage. SDS-PAGE anal. revealed that the lipo-oligosaccharide (LOS) isolated from these **mutants** migrated faster than the wild type LOS and its color changed from black to brown as has been described for E.

coli **htrB mutants**. Immunoblotting anal. also showed that the LOS from the **htrB mutants** lost reactivity to a monoclonal antibody, 6E4, which binds to the wild type NTHi 2019 LOS. Electrospray ionization-mass spectrometry anal. of the O-deacylated LOS oligosaccharide indicated a modification of the core structure characterized in part by a net loss in phosphoethanolamine. Mass spectrometric anal. of the lipid A of the **htrB mutant** indicated a loss of one or both myristic acid substitutions. These data suggest that HtrB is a multifunctional protein and may play a controlling role in regulating cell responses to various environmental changes.

L19 ANSWER 9 OF 10 MEDLINE DUPLICATE 8
AN 95172727 MEDLINE
DN 95172727
TI Molecular cloning and characterization of the nontypeable Haemophilus influenzae 2019 rfaE gene required for lipopolysaccharide biosynthesis.
AU **Lee N G**; **Sunshine M G**; **Apicella M A**
CS Department of Microbiology, University of Iowa, Iowa City 52242..
NC AI 24616 (NIAID)
SO INFECTION AND IMMUNITY, (1995 Mar) 63 (3) 818-24.
Journal code: GO7. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U17642
EM 199506
AB The lipooligosaccharide (LOS) of nontypeable Haemophilus influenzae (NTHi) is an important factor in pathogenesis and virulence. In an attempt to elucidate the genes involved in LOS biosynthesis, we have cloned the rfaE gene from NTHi 2019 by complementing a Salmonella typhimurium rfaE **mutant** strain with an NTHi 2019 plasmid library. The rfaE **mutant** synthesizes lipopolysaccharide (LPS) lacking heptose, and the rfaE gene is postulated to be involved in ADP-heptose synthesis. Retransformation with the plasmid containing 4 kb of NTHi DNA isolated from a reconstituted **mutant** into rfaE **mutants** gave wild-type LPS phenotypes. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis confirmed the conversion of the rfaE **mutant** LPS to a wild-type LPS phenotype. Sequence analysis of a 2.4-kb BglII fragment revealed two open reading frames. One open reading frame encodes the RfaE protein with a molecular weight of 37.6 kDa, which was confirmed by in vitro transcription and translation, and the other encodes a polypeptide highly homologous to the Escherichia coli **HtrB** protein. These two genes are transcribed from the same promoter region into opposite directions. Primer extension analysis of the rfaE gene revealed a single

transcription start site at 37 bp upstream of the predicted translation start site. The upstream promoter region contained a sequence (TA AAAT) homologous to the -10 region of the bacterial sigma 70-dependent promoters at an appropriate distance (7 bp), but not sequence resembling the consensus sequence of the -35 region was found. These studies demonstrate the ability to use complementation of defined LPS defects in members of the family Enterobacteriaceae to identify LOS synthesis genes in NTHi.

L19 ANSWER 10 OF 10 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1995:290502 BIOSIS
DN PREV199598304802
TI Isolation and **mutant** analysis of the **htrb** homologue of the Haemophilus influenzae nontypable strain 2019.
AU **Lee, Na-Gyong; Sunshine, Melvin G.**; Engstrom, Jeffrey; **Gibson, Bradford W.**; **Apicella, Michael A.**
CS University Iowa, Iowa City, IA USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 206.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DT Conference
LA English

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